OLEFIN METATHESIS IN PEPTIDOMIMETICS, DYNAMIC COMBINATORIAL CHEMISTRY, AND MOLECULAR IMPRINTING

By

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This dissertation is dedicated to my parents for their unconditional love and support.

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OLEFIN METATHESIS IN PEPTIDOMIMETICS, DYNAMIC COMBINATORIAL CHEMISTRY AND MOLECULAR IMPRINTING

By

Tammy Karrie Cheng Low

August 2006

Chair: Dr. Eric J. Enholm
Major Department: Chemistry

Catalyzed based olefin metathesis is a very valuable and useful tool in synthetic organic chemistry. Our research goals consisted of employing olefin metathesis in the synthesis of peptidomimetics, and studying the feasibility of this method in dynamic combinatorial chemistry and molecular imprinting of nerve agents.

One of the approaches to the development of peptidomimetics is to attach biologically significant functional groups, such as amino acid side chains, to a scaffold. Grubbs' second generation ruthenium catalyst was used to couple phenylalanine to a 17-membered lactam using cross-metathesis in 48% yield with an *E*:*Z* ratio of 1.2:1. The CM product of two phenylalanine amino acids was isolated in 45% yield and found to be predominantly *trans*. A series of synthetic steps were needed to construct the 17-membered lactam, including the fundamental RCM reaction. The reversibility of the cross metathesis of this macrocyclic system was demonstrated, which is essential to the development of a dynamic combinatorial library

The use of olefin metathesis in dynamic combinatorial chemistry is of interest as a method in generating a library of peptidomimetics. The olefin cross-metathesis reactivity and selectivity of amino acid derivatives with a cyclic scaffold to generate diketopiperizine peptide derivatives were investigated. Product yields were dependent on the amino acid R groups, and whether the amino acid possessed an allyl or homoallyl moiety at the carboxylate side. The stereoselectivity of the dipeptide derivatives was found to be predominantly *trans*. Larger sized cyclic scaffolds were also synthesized to create a more diversified library. In addition, several peptide derivatives possessing diene functionality were examined and subjected to Grubbs' catalyst. Various conditions, such as substrate and catalyst concentrations, as well as different metal templates, were explored.

This research also described a model study to investigate the feasibility of using ring-opening metathesis polymerization in molecular imprinting technology. The goal was to develop a chemical sensor capable of detecting nerve agents. We were able to synthesize the fluoresceinyl dye. In addition, we demonstrated the Michael addition of the thiol compound to a maleimide system and the regeneration of the maleimide, which was required in the development of molecularly imprinted polymers.

CHAPTER 1
HISTRORICAL BACKGROUND

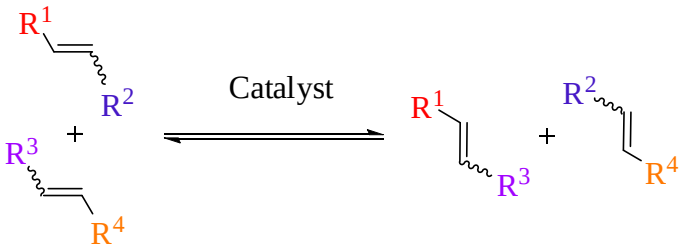
1.1 Olefin Metathesis

Olefin metathesis is a powerful synthetic tool that has found its way into the vast array of science disciplines, from the development of small molecular drug candidates to the industrial sized synthesis of petrochemicals.[1-7] This catalytic organic reaction is unlike other carbon-carbon bond forming strategies due to the versatility of synthetic transformations it promotes, such as the synthesis of various sized cycloalkenes from dienes and specialized polymers by the ring-opening of cyclic molecules. Olefin metathesis has opened efficient synthetic routes to complex natural products, medicinal drugs, and new materials as demonstrated by the explosion of metathesis related applications found in literature during the past decade. In 2005, the value of this organic reaction was prestigiously recognized by the award of the Nobel Prize in Chemistry to the major contributors of olefin metathesis – Yves Chauvin, Robert H. Grubbs, and Richard R. Schrock.

1.1.1 Development of Olefin Metathesis and Catalysts

Olefin metathesis was first discovered accidentally by researchers in petrochemical companies in the 1950s when they were searching for heterogenous catalyst to covert olefins to high-octane gasoline.[7,8] Instead of their expected products, the chemists observed newly formed olefins. It wasn't until the 1960s when researchers at Goodyear Tire & Rubber determined that these new products were the result of an exchange of

substituents on different olefins, which they officially referred to as “olefin metathesis”[9] as shown in Scheme 1-1.

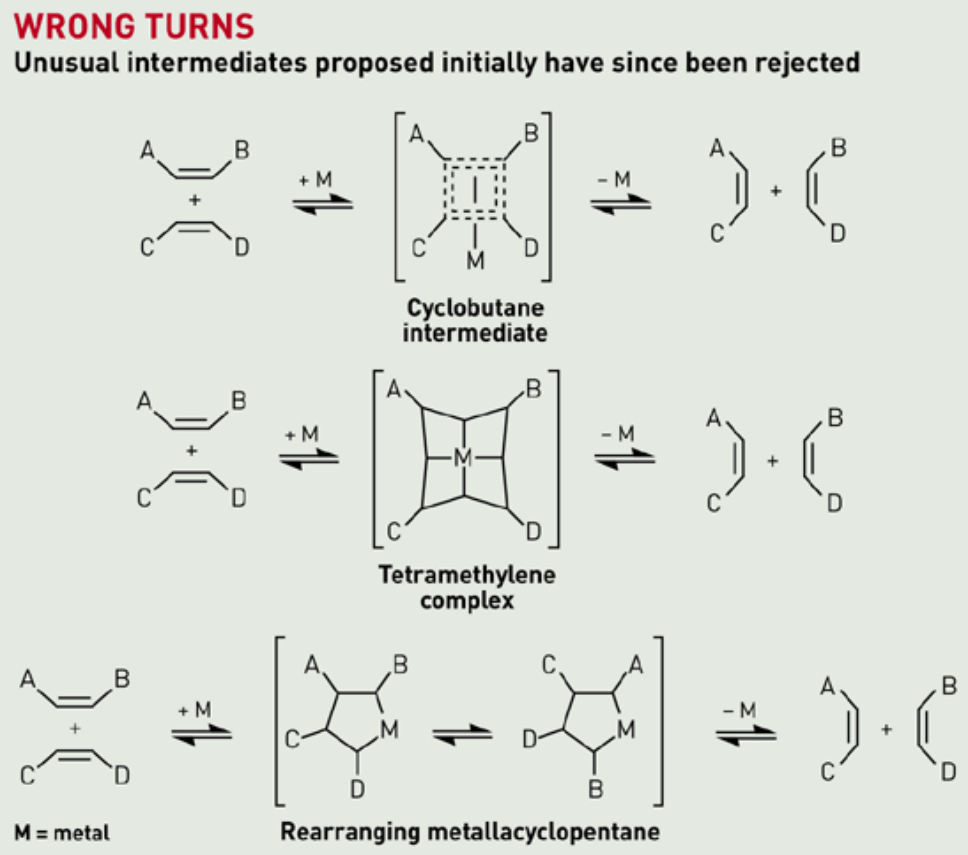


Scheme 1-1. Olefin metathesis

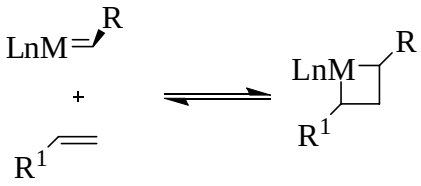
For several years, chemists attempted to explain the mechanism behind the novel reaction for the skeletal transformation of olefins. Calderon,[10] Pettit,[11] and Grubbs[12] initially suggested cyclobutane, tetramethylene complex, and rearranging metallacyclopentane intermediates as part of the mechanism, respectively, but all proposals were later proved to be incorrect (Scheme 1-2).[8] It was in 1971 when French chemist Yves Chauvin proposed a metal-carbene mechanism which involves the formation of a metallacyclobutane intermediate (Scheme 1-3).[13] However, the debate over the mechanism continued for years until Katz, Schrock, and Tebbe independently conducted experiments which supported Chauvin’s proposal and is now widely accepted by the community.[1,8]

During the debate over the olefin metathesis mechanism, several groups continued to develop transition metal carbene complexes, including the Fischer carbenes (low oxidation state metals and electron poor at the carbon center) and the Schrock carbenes (high oxidation state metals and electron poor at the metal center).[1,8] The Fischer carbenes showed little activity for olefin metathesis, and Schrock’s early tantalum and niobium complexes proved unsuccessful as well.[1,8] However, these initial studies paved

the road to improved alkylidene complexes that eventually demonstrated improved reactivity for olefin metathesis.



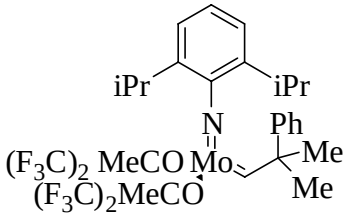
Scheme 1-2. Proposed intermediates for olefin metathesis



Scheme 1-3. Cyclobutane intermediate proposed by Chauvin

Despite early advances in catalyst developments, olefin metathesis was not a practical synthetic methodology because of the catalysts' low reactivity, lack of stability and tolerance towards functional groups. It wasn't until the 1990's when Schrock introduced the well-defined alkoxy imido molybdenum-based catalyst **1-1**, which made olefin metathesis useful (Figure 1-1).[14,15] In contrast to the early catalyst systems of the

1970s and 1980s, which are often referred as “classical” or “ill-defined” catalysts because the propagating species can not be observed, isolated, or structurally characterized, the molybdenum alkylidene complex is highly reactive and leads to desired products in high yields, even with sterically hindered alkenes.[1,16] However, the downfall is the catalyst’s relatively limited tolerance toward polar functional groups such as alcohols and carboxylic acids, and its sensitivity to air and moisture.[5]

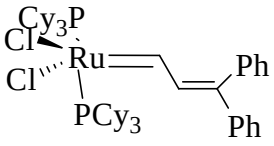


Shrock's catalyst
1-1

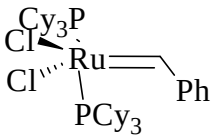
Figure 1-1. Alkoxy imido molybdenum-based catalyst **1-1**

In an effort to improve tolerance for functional groups and moisture, Grubbs and coworkers examined ruthenium catalysts, which have an oxidation state lower than Schrock’s mettalolefins but higher than the Fischer carbenes.[1,17] Despite the development of ruthenium catalyst [$(PPh_3)_2Cl_2Ru{=}CHCH{=}C(Ph)_2$] (**1-2**) in 1992, which was stable in protic and aqueous solvents, the catalyst exhibited limited reactivity compared to Schrock’s carbene complexes (Figure 1-2).[1,18,19] Modifications of the ruthenium catalyst were conducted throughout the years and eventually in 1996, “Grubbs’ first generation catalyst” **1-3** was introduced, which not only displayed functional group tolerance but also 20-10,000 times more activity than ruthenium catalyst **1-2** (Figure 1-2).[20] In 1999, based on Herrmann’s studies on N-heterocyclic carbenes,[21] Grubbs substituted one of the tricyclohexyl phosphine (PCy_3) ligands from **1-3** with a

mesityl N-heterocyclic ligand to afford the more stable ruthenium complex **1-4**, which is now referred as "Grubbs' second generation catalyst" and is far superior to other catalysts because of its tolerance to air, moisture, and a wide variety of functional groups (Figure 1-2).[1,6,22,23] As a result of these enhanced properties, our research efforts focused on olefin metathesis utilizing Grubbs' second generation catalyst **1-4**.

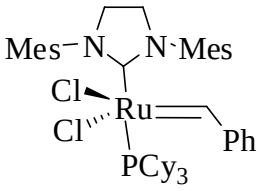


1-2



Grubbs' first generation catalyst

1-3



Grubbs' second generation catalyst

1-4

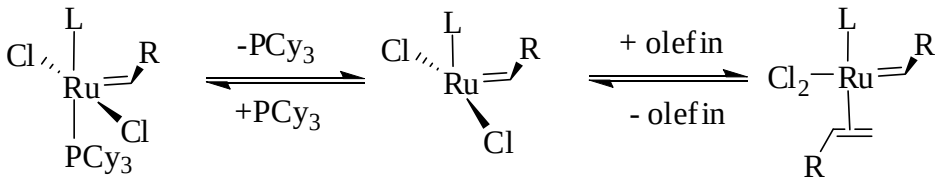
(Cy = cyclohexyl, Mes = 2,4,6-trimethylphenyl)

Figure 1-2. Ruthenium catalysts

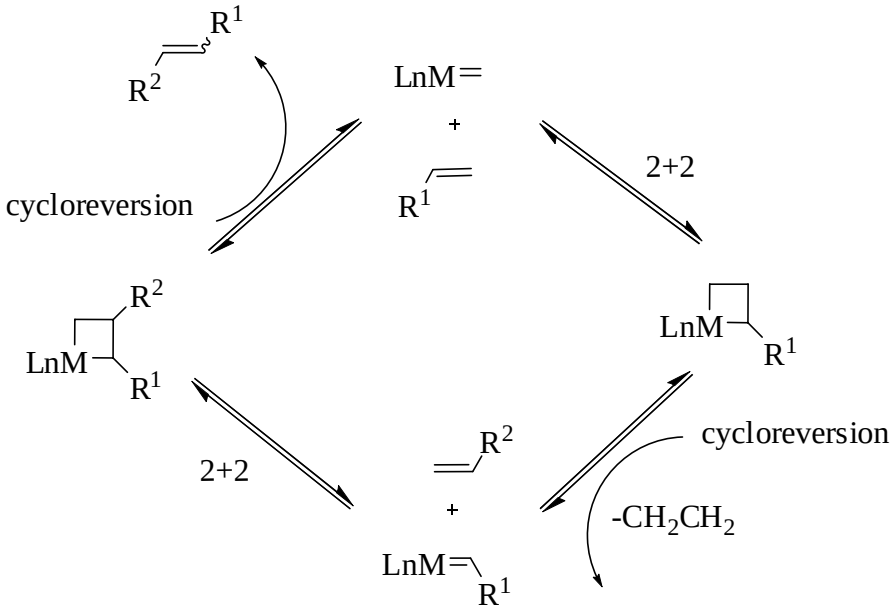
1.1.2 Mechanism of Olefin Metathesis

The commercial availability of ruthenium catalysts **1-3** and **1-4** has made them a practical, standard organic tool, thus the synthesis of the metal alkylidene complexes will not be discussed. However, to better apply olefin metathesis towards the synthesis of target compounds and polymers, it is helpful to examine the mechanism that was first introduced by Chauvin. When utilizing Grubbs' catalysts **1-3** and **1-4**, the first step of the mechanism involves the dissociation of the PCy_3 ligand, followed by the binding of the alkene to the carbene (Scheme 1-4).[24,25] The next step is a 2+2 cycloaddition with the metal catalyst to form the metallabutane intermediate, which can then undergo a cycloreversion to produce a new metal alkylidene (Scheme 1-5).[4,25] The mechanism proceeds as a catalytic cycle where the metal alkylidene undergoes another 2+2

cycloaddition with a second alkene, followed by the cycloreversion leaving the newly formed olefin with R^1 and R^2 groups and the metal alkylidene for further catalytic use.



Scheme 1-4. Dissociative substitution of ruthenium catalysts



Scheme 1-5. Mechanism of olefin metathesis

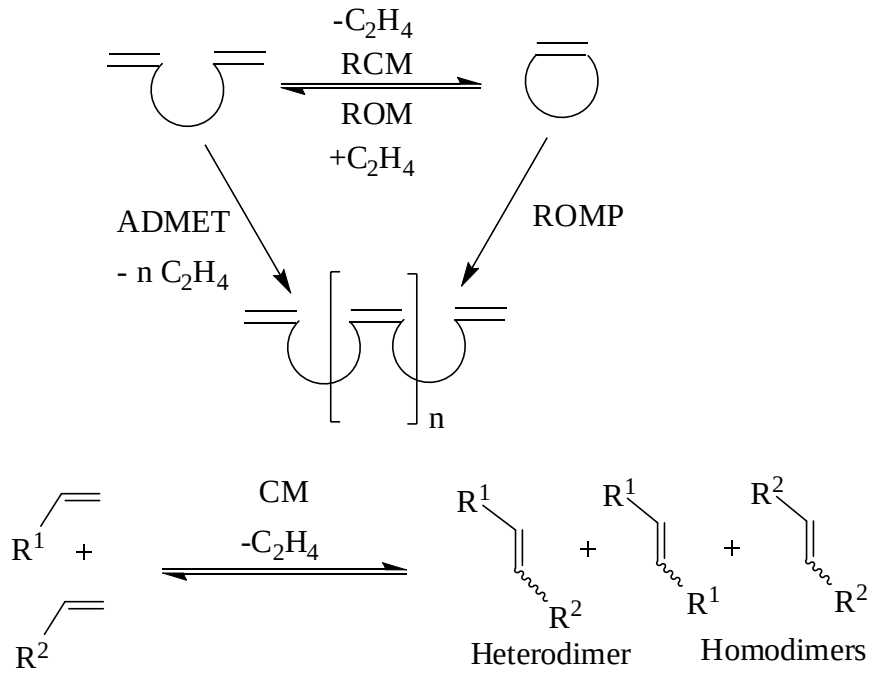
Because ethylene gas is released as a byproduct,[6] it is possible to shift the equilibrium toward the desired products by deliberately evacuating or flushing the headspace with argon to remove ethylene.[26] The cycle continues until the reaction is quenched, for example, with ethyl vinyl ether (EVE) which reacts with the ruthenium catalyst and forms the Fischer carbene $L(PCy_3)(Cl)_2Ru{=}CHOEt$, thus eliminating the catalysts reactivity.[24]

1.1.3 Important Types of Metathesis Reactions and Applications

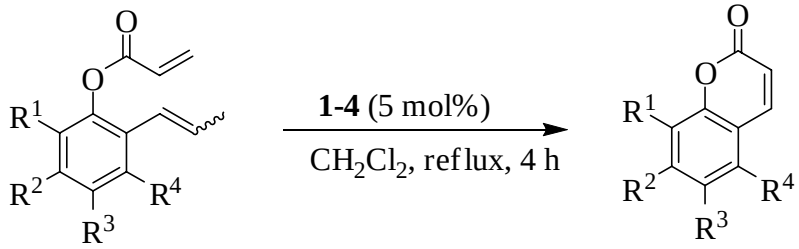
As highlighted many times, olefin metathesis is a versatile technique which includes ring-closing metathesis (RCM), ring-opening metathesis (ROM), cross-metathesis (CM), ring-opening metathesis polymerization (ROMP) and acyclic diene metathesis (ADMET) (Scheme 1-6).[6] The three main metathesis reactions used in our studies, RCM, CM and ROMP, will be discussed in greater detail.

RCM is the cyclization of a diene to generate various sized cycloalkenes, from small 5-membered rings to macrocycles.[16] The stereochemistry of the cycloalkene products are dependent on the substrates; for example, small and medium sized rings formed from RCM are in a less strained *cis* conformation while in contrast, the stereochemistry of non-rigid RCM derived macrocyclic compounds is difficult to predict and can encompass a mixture of *cis* and *trans* stereoisomers.[27]

RCM reactions are conducted under highly dilute conditions to prevent ADMET polymerization. In addition, heat is often employed to improve ring closures due to the entropy of activation required to bring the two ends of the chain together.[28] However, higher temperatures can cause the catalyst to decompose, thus a greater catalyst loading is required.[5] Despite this requirement, RCM has provided a shorter, more efficient synthetic route to natural products, medicinal drugs, and new materials, compared to conventional methods, as attested by the numerous studies found in literature. An example is shown in Scheme 1-7 in which Van and coworkers utilized RCM to synthesize coumarins in excellent yields,[29] while other methods reported in literature had disadvantages and required harsher conditions.



Scheme 1-6. Types of olefin metathesis



1-5a R^1 = OMe; $R^2 = R^3 = R^4$ =H
1-5b $R^1 = R^2 = R^4$ = H; R^3 = OMe
1-5c $R^1 = R^4$ = H; $R^2 + R^3$ = O-CH_2-O
1-5d $R^1 = R^4$ = OMe; $R^3 = R^2$ =H
1-5e $R^1 = R^4 = R^3 = R^2$ = H

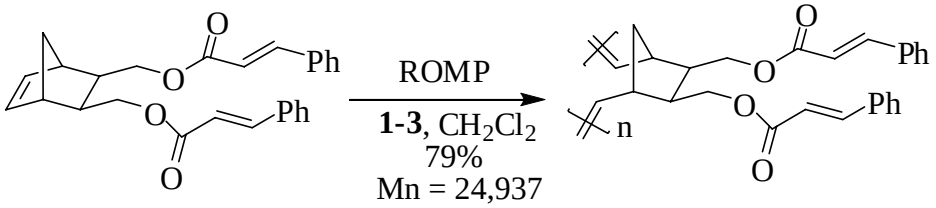
1-6a 81%
1-6b 90%
1-6c 83%
1-6d 72%
1-6e 70%

Scheme 1-7. Utilizing RCM to synthesize coumarins

The reverse reaction of a RCM is ROM, where the cycloalkene breaks open to form two terminal dienes, which can be followed by a CM reaction with other acyclic alkenes to form new products.[5] Similar to RCM, ROM requires dilute conditions due to the resulting dienes undergoing polymerization, referred to as ROMP. The polymerization is quite practical in the synthesis of specialized polymers and is more widely used than

ROM. Cycloalkenes which possess ring strain, such as norbornene, cyclopentene, and cyclooctene, favor ROMP.[16] By reducing ring strain, the reaction is enthalpically driven forward, and is not reversible.

Grubbs' catalyst **1-4** has high functional group tolerance and has been demonstrated in ROMP to generate functionalized, telechelic, and trisubstituted polymers.[30] ROMP is responsible for the synthesis of a variety of new materials, from the development of nonlinear optics to biologically relevant polymers.[31]. A recent application of this polymerization is shown in Scheme 1-8, where a polymer was synthesized to create biomaterials that can under go a 2+2 cycloaddition when irradiated with UV light.[32]

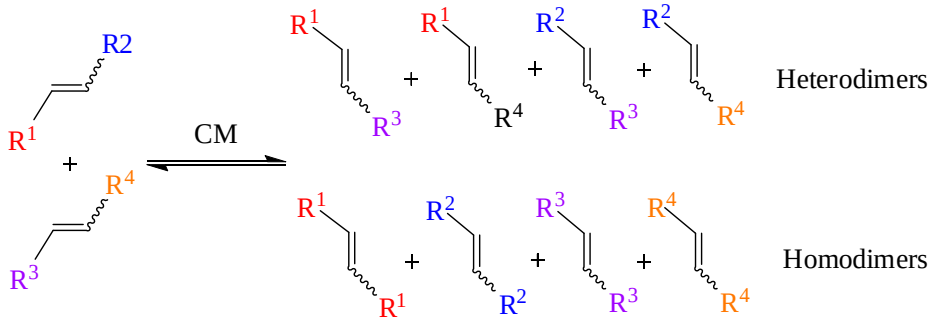


1-7 **1-8**

Scheme 1-8. Employing ROMP to create new materials

RCM and ROMP started as the most popular types of metathesis reactions, but due to recent studies and a better understanding of the selectivity and stereoselectivity of CM, it has become a more useful and versatile synthetic technique over the years. The concerns over selectivity arose from the mixture of heterodimers, homodimers, *cis* and *trans* stereoisomers that can be generated from CM reactions. In addition, employing asymmetric internal olefins in CM can also lead to a greater number of product mixtures (Scheme 1-9). Factors such as sterics and electronic effects may also affect CM reactivity and selectivity, and must be considered when planning reactions. For example,

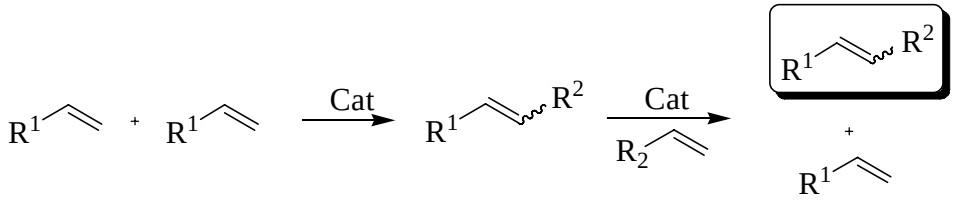
olefins possessing electron withdrawing or bulky substituents often leads to little or no CM products because of the poor reactivity with the catalyst, but steric effects can favor *trans* selectivity [33]



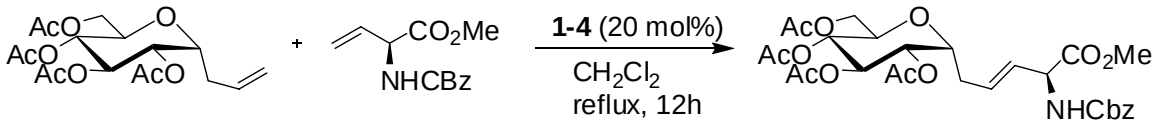
Scheme 1-9. CM of asymmetric internal olefins

Fortunately, new models and methodology were developed to improve selective CM.[26,33,34] For instance, Grubbs *et al.* categorized olefins as Type I, II, III, and IV based on their reactivity to form homodimers by CM with catalyst **1-3** and **1-4**. Primary allylic alcohols, protected amines and esters are examples of Type I alkenes (sterically unhindered, electron-rich) because they readily form homodimers by CM and also undergo secondary metathesis reactions. The more sterically hindered Type II alkenes (i.e. secondary alcohols and vinyl ketones) are less reactive, and Type III alkenes are nonreactive (i.e. tertiary allylic carbons). Type IV alkenes (i.e. protected trisubstitued allyl alcohols) are spectators and do not participate in the CM reaction. The examples given above are based on the utilization of catalyst **1-4**. One strategy toward selective CM involves a two step procedure in which homodimers of Type I alkenes are generated, followed by a secondary metathesis reaction with Type II/III alkenes to preferentially form the heterodimer product with *trans* favored in the presence of selected functional groups(Scheme 1-10).[33] CM is more widely used now, and an example of a recent application of CM is shown in Scheme 1-11, where Nolen and coworkers were

able to synthesize C-glycosyl asparagines in good yields with predominantly *trans* selectivity.[35]



Scheme 1-10. Primary and secondary CM metathesis reactions



1-9 **1-10** **1-11** 82%

Predominantly *E*

Scheme 1-11. Synthesis of C-glycosyl asparagines via CM

1.2 Peptidomimetics

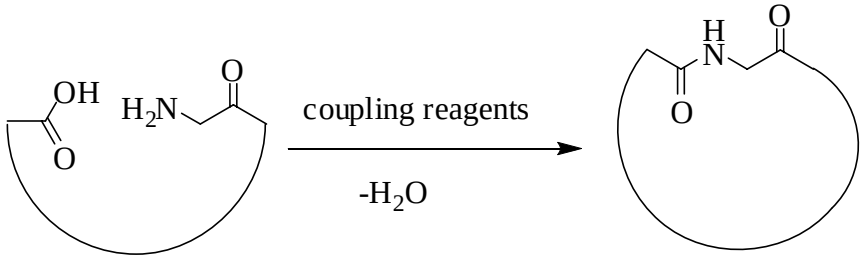
Olefin metathesis is now a common synthetic tool for the organic chemist. As shown by the examples in the previous section, it is versatile and has been used to make new materials and analogues of natural products. Another area of science that has a particular interest in metathesis is peptide and medicinal chemistry. Peptides, which are made up of amino acids, are vital biological molecules with vast functionality. For example, they can serve as antibiotics, pain relievers, and building blocks of important proteins, such as enzymes, which possess a variety of hormonal activities. Therefore, there is a significant interest in peptides as drug candidates. Unfortunately, natural peptides typically don't make good pharmaceutical drugs because of their lack of bioavailability and susceptibility to hydrolysis.[36,37] A solution to this downfall is peptidomimetics, which are synthetic molecules that can mimic the peptide's topography and functionality, but possess improved biological and pharmaceutical properties.[38,39]

One strategy to enhance these properties is by synthesizing cyclic molecules that mimic peptides, which are referred as cyclic peptidomimetics. The frequent discovery of natural cyclic peptides possessing antibiotic, antiviral, antitumor, and therapeutic properties has made them an active area of research.[40,41] In addition, cyclic peptides are more stable than linear peptides because of their constrained conformation, which improves receptor selectivity. [36] Therefore, there is great interest in the synthesis of mimetics of cyclic peptides for use in medicinal chemistry and drug development. Peptidomimetics can be designed to be less susceptible to proteolysis, and biostable with improved ability for absorption in the body.[42,43]

There are several methods to synthesize cyclic amino acids and peptidomimetics. One strategy involves cyclization of a linear peptide by conventional coupling agents to form a new amide bond (Scheme 1-12)[36,44] Some common reagents are dicyclohexylcarbodiimide (DCC), diisopropylcarbodiimide (DIC), and expensive reagents such as HATU or PyBroP, which are more efficient.[44] Racemization of the chiral center is of great concern, and often times racemization suppresants such as 1-hydroxy-7-aza-benzotriazole (HOAt) and 1-hydroxybenzotriazole (HOBt) must be employed.[44] Cyclization can sometimes be complicated due to the difficulty in bringing the two terminal ends together,[41] thus peptidomimetics are often designed where hydrogen-bonds can assist in the ring closure by inducing the linear peptide to turn (Figure 1-3).[36]

It is also possible to synthesize cyclic peptidomimetics by attaching pharmacophoric groups, such as amino acids, to a scaffold.[42,43,45,46] The scaffold can be designed where the functional groups are properly oriented to their corresponding

binding sites. For example, Murphy and coworkers synthesized potential HIV protease inhibitors by grafting pharmacophoric groups on a azasugar scaffold (Figure 1-4),[46] but a rather lengthy synthetic scheme starting from D-Fructose and involving selective deprotection was required. Other types of scaffolds have been generated including conformationally constrained bicyclic amino acid motifs[38] and pyridine derivatives.[43]



linear peptide cyclic peptide

Scheme 1-12. Cyclization of a linear peptide using coupling agents

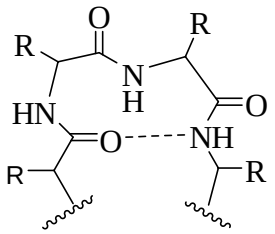
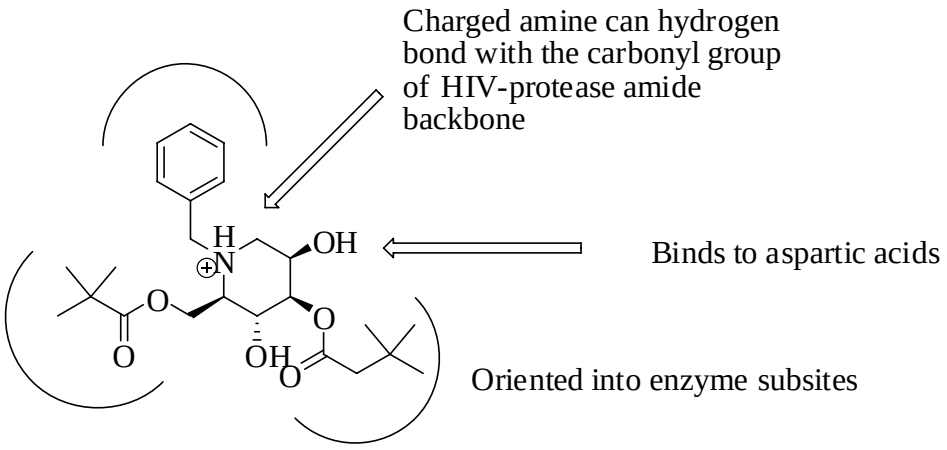


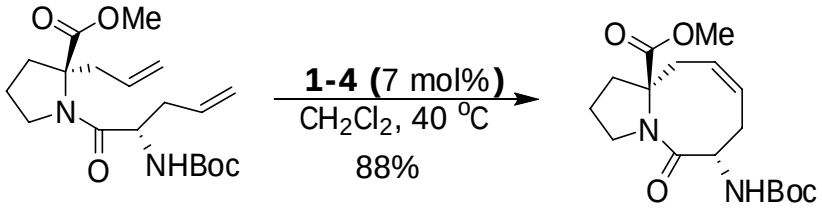
Figure 1-3. Hydrogen-bonding leads to bent conformation

The examples above give a brief overview of peptidomimetics and some of the strategies used to generate cyclic mimetics. Many of the examples used to synthesize scaffolds and cyclic involve intricate synthetic procedures. Recently, RCM has been utilized as an alternative method towards generation of cyclic peptidomimetics.[36,47,48] An example is shown in Scheme 1-13, where Gmeiner and coworkers employed RCM toward the synthesis of β-turn mimetics in excellent yields.[49]



1-12

Figure 1-4. Design of putative azasugar peptidomimetic



1-13 **1-14**

Scheme 1-13. Use of RCM toward the synthesis of β-turn mimetics

1.3 Dynamic Combinatorial Chemistry

Besides applications in peptidomimetics, olefin metathesis in dynamic combinatorial chemistry (DCC) is very promising. DCC has gained interest in recent years as a powerful methodology for exploring molecular recognition systems, and lead to the discovery of new biological molecules, drugs, receptors, and catalysts.[50,51] A dynamic combinatorial library (DCL) is made from molecular building blocks connected via reversible linkages which interconvert in a thermodynamic equilibrium.[50,51] A template, such as a biological molecule, is added to the system which can shift the equilibrium to form one major constituent in the library (Figure 1-5).[52] Unlike traditional combinatorial techniques, where molecules are created en masse and tested for desired

properties, DCC combines the synthesis and selectivity of library constituents in one pot. Some key differences between DCC and traditional combinatorial chemistry are shown in Table 1-1. DCC is not only valuable in discovering new properties, but also as a learning tool towards a better understanding of molecular recognition, self-assembly, and supramolecular chemistry.

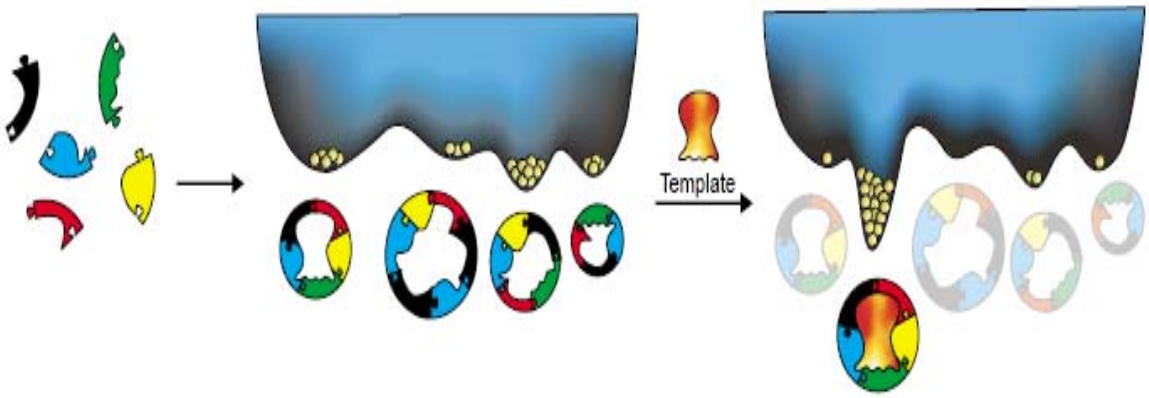


Figure 1-5. A dynamic combinatorial library and its free energy landscape

Table 1-1. Differences between traditional and dynamic combinatorial libraries

COMBINATORIAL LIBRARY	DYNAMIC COMBINATORIAL LIBRARY
Real set	Virtual set
Collection of molecules	Collection of components
Covalent	Covalent or non-covalent
Non-reversible	Reversible
Systematic	Recognition-directed
Preformed by synthesis	Self-assembled
In absence of target	In presence of target

There are two types of dynamic combinatorial recognition as described in the biological aspects of receptors and substrates in Figure 1-6.[51,53] In the casting process, the target receptor (T_R) influences the organization of the building blocks into the substrate with specific binding affinity to the T_R. In the molding process, the target molecule is a substrate (T_S) which results in the molding of the receptor.

The above example shows the addition of the target molecule to the reaction mixture after the library of building blocks have assembled. The presence of the target can then shift the equilibrium where only the constituents with the highest affinity for the target will be amplified. This is considered a true dynamic combinatorial system.[54] The other method, which is sometimes referred as the generation of a virtual combinatorial library, involves the addition of the target molecule without formation of a library of interchangeable species,[54] as depicted by the lock and key metaphor shown in Figure 1-7.[54]

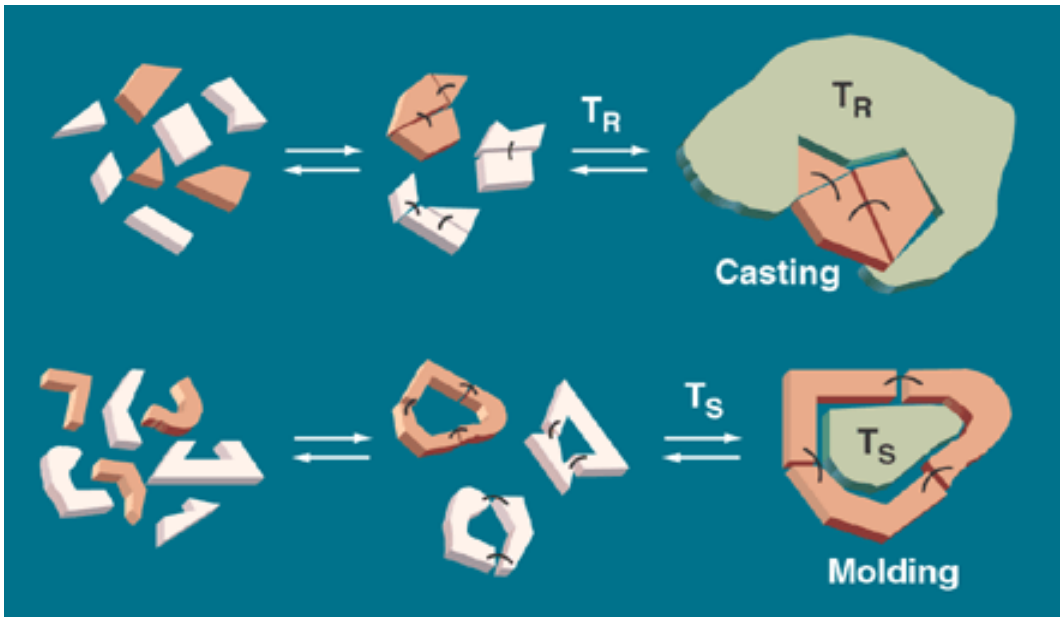


Figure 1-6. Casting and molding process in dynamic combinatorial chemistry

There are different types of reversible reactions that can potentially be used in DCC, such as covalent bond formation, interactions, and intramolecular processes (Table 1-2)[54] Some reversible reactions that have already been studied for use in DCC include disulfide exchange,[55] metal-ligand coordination [56], exchange of oximes[57] and hydrazones[58], and olefin metathesis.[59,60]

Even with the expanding amount of dynamic combinatorial related research found in literature, very few studies have examined olefin metathesis in DCC. One of the most interesting developments came from Nicolaou's group in 2000, in which they proved the applicability and significance of dynamic combinatorial chemistry via olefin metathesis by synthesizing dimers of vancomycin derivatives[61] (Scheme 1-14). The building blocks possessed various olefin chain lengths and different amino acid R groups, while the amino acid target chosen had a high affinity to vancomycin. As they expected, the dimers with the shorter tethers were amplified upon addition of the target[61] (Figure 1-8). Despite Nicolaou's important studies on DCL of vancomycin derivatives, there is still more to learn about the reactivity, influence of functional groups, and stereochemistry of olefin metathesis reactions for use in DCC.

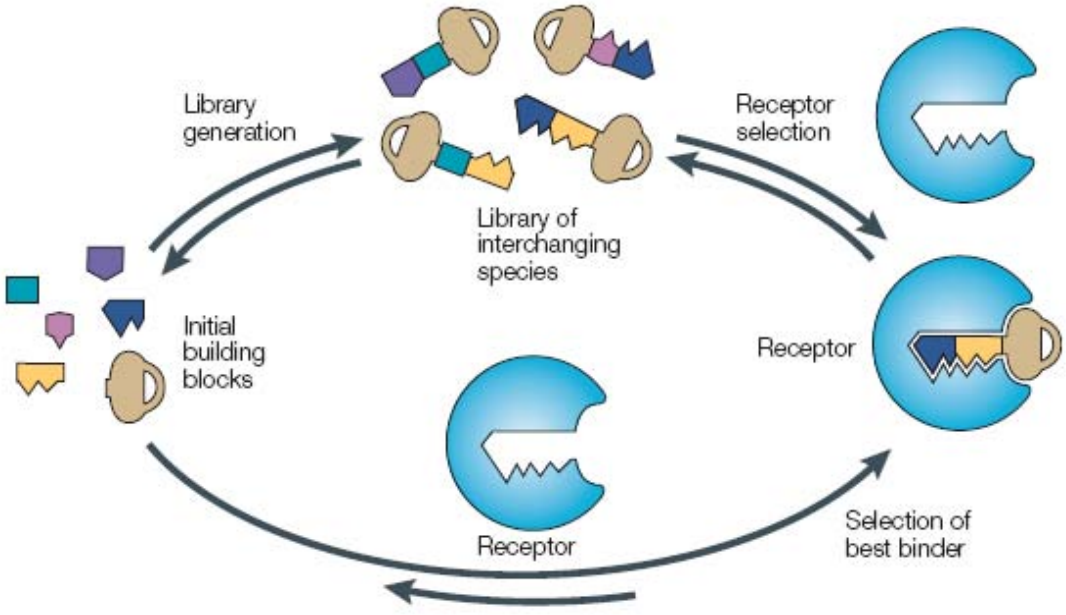
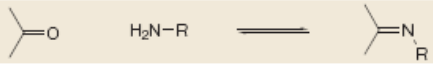
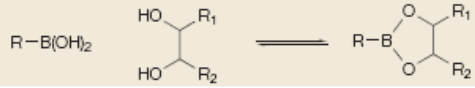
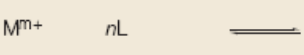
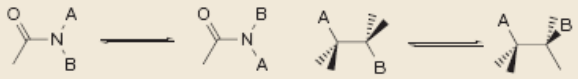
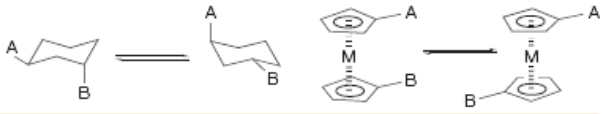
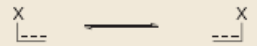
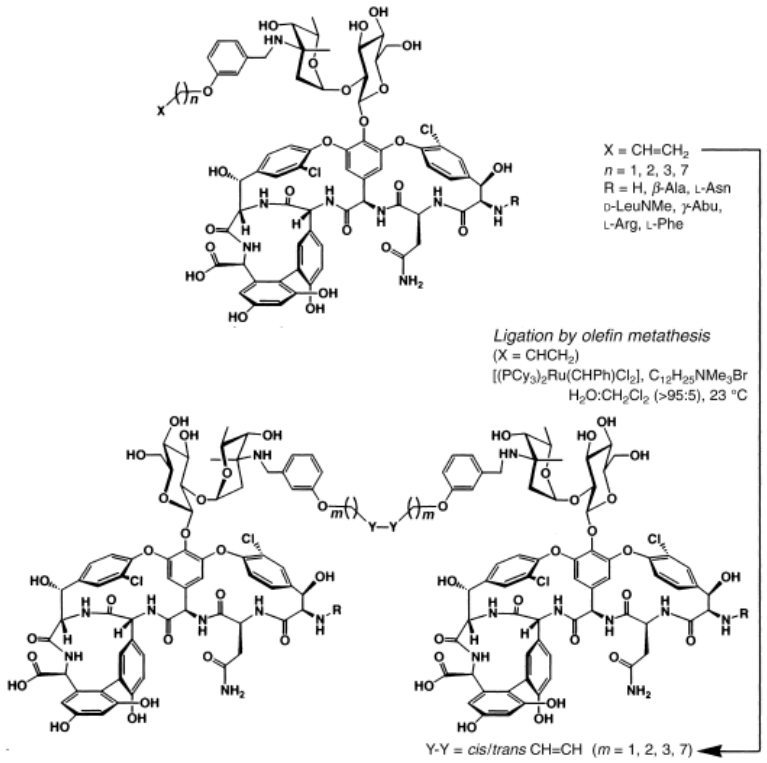


Figure 1-7. Dynamic combinatorial chemistry (top) versus virtual combinatorial libraries (bottom)

Table 1-2. Dynamic process for potential use in DCC systems

Reversible covalent bond formation	
Carbonyl reactions	
Imine formation	
Hemiketal formation	
Transacylation	
Aldol formation	
Michael reaction	
Disulphide formation	
Diels–Alder reaction	
Metathesis reaction	
Boronic ester formation	
Reversible interactions	
Metal coordination	
Electrostatic interaction	
Hydrogen bonding	
Donor–acceptor interaction	
Reversible intramolecular processes	
Configurational	
Cis–trans isomerization	
Conformational	
Internal rotation	
Ring inversion	
Structural	
Tautomerism	
Fluxionality	



Scheme 1-14. Dimerization of monomeric vancomycin derivatives with terminal olefins by olefin metathesis

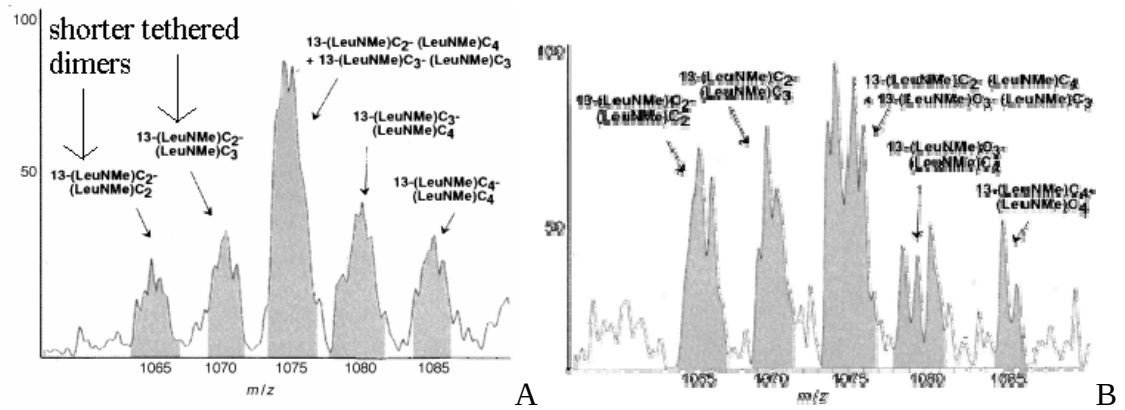


Figure 1-8. Mass spectrometric analysis of the vancomycin dimer mixture in the (A) absence of a target and (B) with the presence of a target.

1.4 Molecular Imprinted Polymers

There has been a great interest in the Enholm group to employ ROMP in molecular imprinted technology, which has only been reported once in literature by Steinke.[62]

Before discussing the strategies of ROMP in the synthesis of molecular imprinted polymers (MIPs), a brief overview on this technology needs to be discussed

Molecular imprinted polymers (MIPs) are useful as mimics of biological receptors, enzymes, and antibodies,[63-68] and can be designed as drug delivery or drug separation systems.[65] In addition, research has already shown that MIPs can have enhanced catalytic activities than the corresponding natural catalytic antibodies.[68] Although a plethora of studies found in literature are related to biological applications, this technology has also been used for chemical sensors[69,70] and environmental analysis.[69]

The general principle behind molecularly imprinted technology involves the synthesis of a polymer that possesses a 3-dimensional molecular memory or "imprint" for a target. For example, in the lock and key metaphor by Emil Fischer, an enzyme ("lock") has active sites specific for a particular substrate ("key") (Figure 1-9). In MIP, the polymer acts as an enzyme by having a cavity with functional groups that can interact covalently or non-covalently with the template (Figure 1-9). Like the enzyme, the polymer has a specific binding affinity for the template.

The general process of molecularly imprinted technology is shown in Figure 1-10,.[67] in which a template and functional monomers, which include a polymerizable moiety, interact to form a stable template-functional monomer assembly. A cross-linker is added and polymerization proceeds resulting in a rigid template-cross linked polymer complex. The template is then extracted to give the MIP.

There are two main ways a template can interact with the functional monomers – the self-assembly approach via non-covalent (i.e. electrostatic, hydrogen bond, and hydrophobic) or metal co-ordination interactions, and the pre-organized approach via

reversible covalent interactions,[67] where both have their advantages and disadvantages. With the pre-organized approach, the template is fixed in its proper orientation during polymerization resulting in a pronounced imprint.[63] However, only selected reversible covalent reactions, such as the formation and hydrolysis of boronate esters, are suitable for MIP.[66,70] An example of the pre-organized approach is shown in Scheme 1-15, in which Wang and coworker built fluorescent sensors using boronate esters.[71]

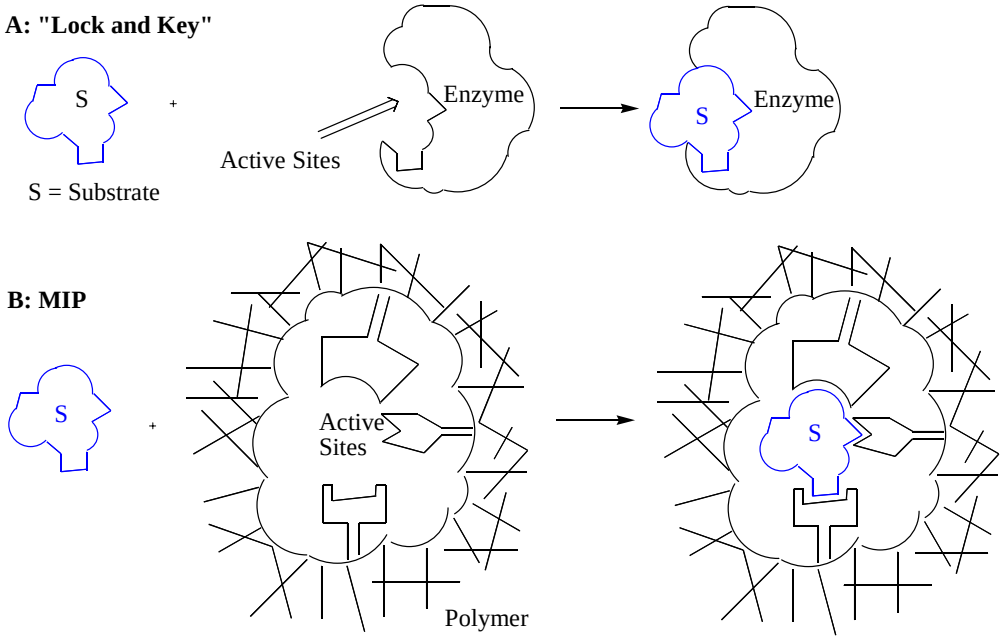


Figure 1-9. A comparison between the A) Lock and Key model and the B) MIP model

The non-covalent approach is the most widely used because of the ease of removing the template by breaking the non-covalent interactions.[65] However, more functional monomers are required to fix the template in place prior to polymerization. If the template is not properly set, then the resulting molecular imprint could have a reduction in the specificity for the template. A solution to both methods is a semi-covalent approach, in which the template is covalently bound during imprinting and non-

covalently bound during rebinding.[70] For example, Whitcombe *et al*. designed a molecular imprint of a tripeptide using this semi approach, as shown in Scheme 1-16.[72]

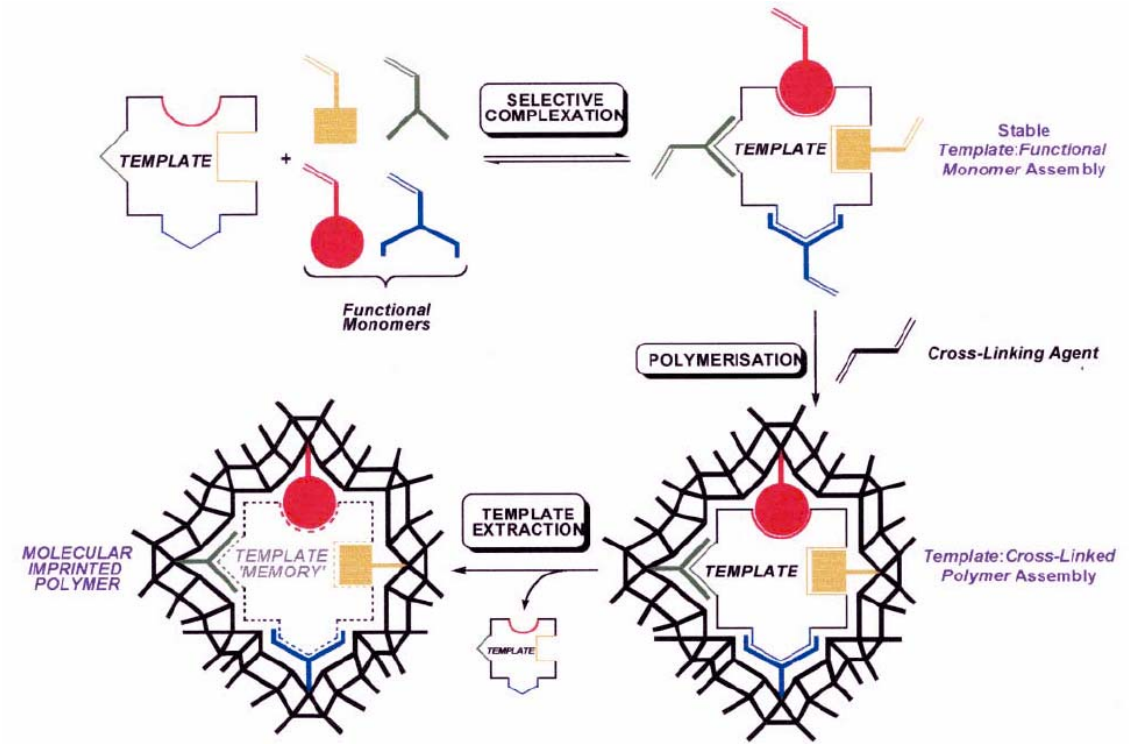
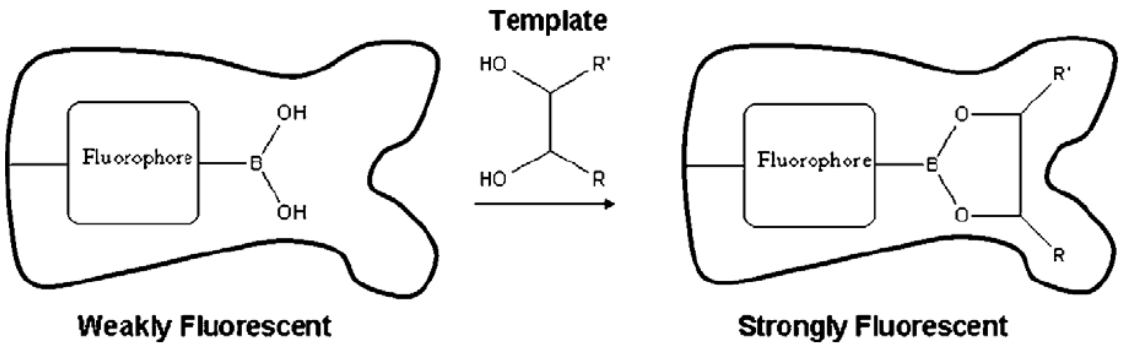


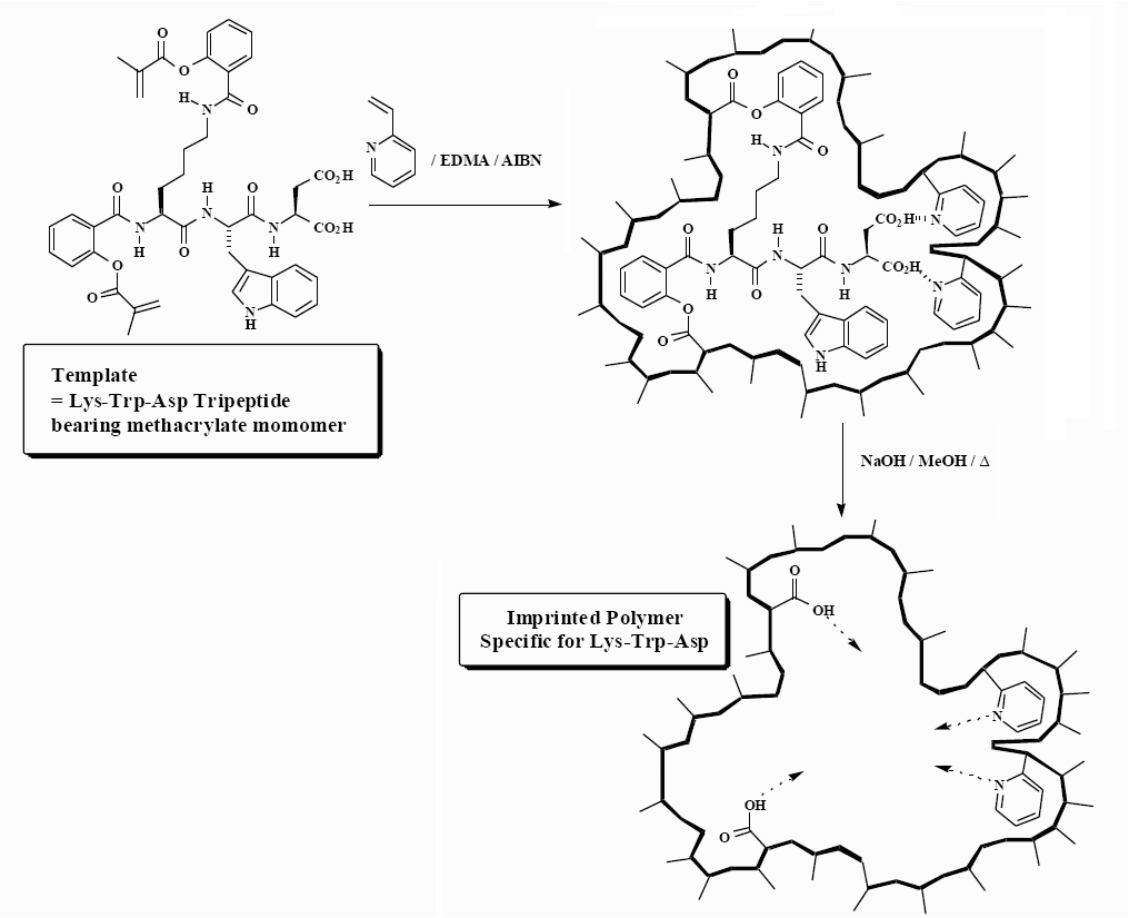
Figure 1-10. A general process for molecular imprinting



Scheme 1-15. An example of the self-assembly approach via covalent interactions

Radical polymerization is the standard method for the synthesis of MIPs, where the functional and cross linking monomers chosen for MIP often possess vinyl or acrylic groups, such as methylmethaacrylate (MMA) and ethylene glycol dimethacrylate

(EDMA). A large variety of these monomers are readily available and have functional groups capable of hydrogen bond, hydrophobic, and other interactions with the template.[64] The radical polymerization can proceed with thermal initiators (azo-*bis*-isobutyronitrile (AIBN) most commonly used), or photoinitiators.[70]



Scheme 1-16. An example of a semi-covalent approach to MIPs

In 2003, Steinke *et al*. used ROMP rather than radical polymerization to synthesize enantioselective MIPs to thermodynamically control the polymerization reaction.[62] The goal was to prevent the formation of MIPs polyclonal cavities, which can decrease selectivity for the template. This was the first time ROMP was demonstrated in generation of MIPs. During that same time period, Allias performed a side by side comparison of radical polymerization and ROMP employing Grubbs' **1-4** catalyst, and

discovered that ROMP required shorter reaction times and easier work-up conditions.[73] Preliminary studies also showed that there was an increase in the affinity for the template using the ROMP strategy, while the MIP cavity generated by radical polymerization showed poor selectivity for the template. Another benefit of employing ROMP is that heat or photo labile templates which are not acceptable in radical polymerization can now be used in the system. Based on these initial studies, it appears promising to examine olefin metathesis in molecularly imprinted technology.

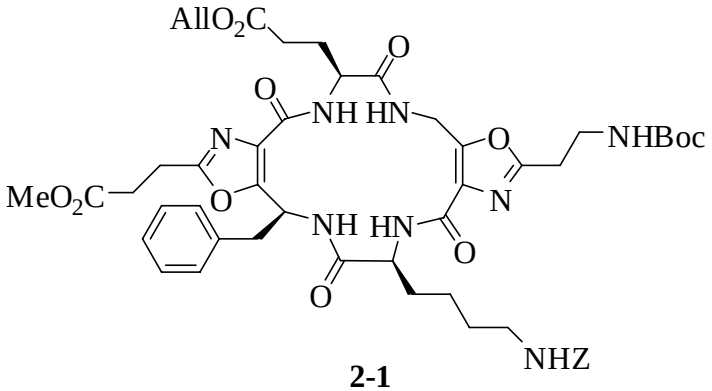
Olefin metathesis is a powerful organic synthetic tool, as attested by the large volume of metathesis related research found in literature. Grubbs' second generation catalyst **1-4** and its tolerance for functional groups have made this methodology even more useful. However, there are still areas of olefin metathesis that require more studies in the field of peptidomimetics and MIPs. The work presented here will examine the use of olefin metathesis in the 1) attachment of a pharmacophoric group on a macrocyclic scaffold by CM; 2) synthesis of dipeptide mimetics by CM and the examination of the reactivity, stereochemistry, and feasibility of CM in dynamic combinatorial chemistry; 3) generation of a dynamic combinatorial library of macrocyclic compounds employing peptidomimetic dienes and 4) development of chemical sensors using ROMP in molecularly imprinted technology to detect nerve agents.

CHAPTER 2
USE OF CROSS-METATHESIS TO COUPLE L-PHENLYALANINE TO A MACROCYCLIC LACTAM

2.1 Introduction

Peptidomimetic research is of paramount importance to the field of medicinal chemistry. One approach toward the synthesis of peptidomimetics is to use a molecular template or a scaffold to which important pharmacophoric groups, such as amino acid side chains, are covalently anchored.[26,30,33,62] These molecules have an excellent potential for chiral discrimination and display stabilization of functional groups.

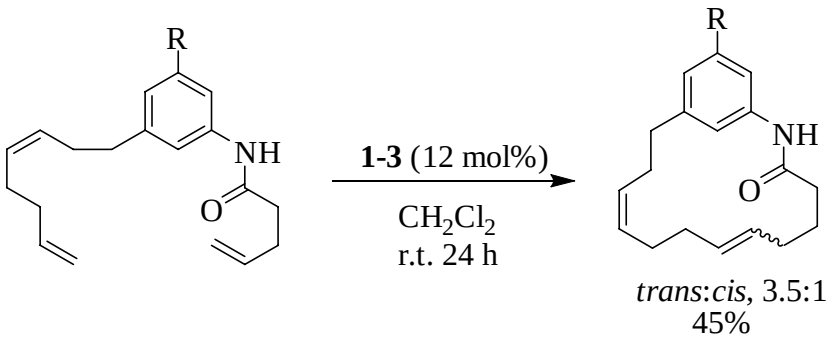
Macrocyclic lactams are excellent choices as scaffolds because many naturally occurring macrocycles possess important biological and medicinal properties.[74,75] In addition, the ability to attach multiple functional groups on a large ring can increase diversity, which is essential in drug developent.[76] One example of a macrocyclic scaffold used for drug discovery is shown in Figure 2-1, in which side chains are attached to a rigid macrocyclic lactam in a well-defined orientation toward receptor sites.[77]



2-1

Figure 2-1. Oxazole-based macrocyclic lactam

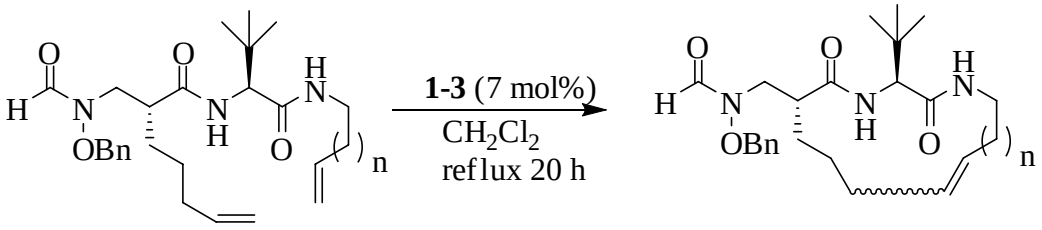
Ring-closing metathesis (RCM) has recently become a useful organic reaction to synthesize macrocylic scaffolds as demonstrated in Scheme 2-1.[75] In this example, Blagg utilized Grubbs' first generation catalyst **1-3** to generate an analogue of Trienomycin A, an antibiotic that also possesses antitumor activity.[75]



2-2 **2-3**

Scheme 2-1. Synthesis of an analogue of Trienomycin A utilizing RCM

Another example of metathesis derived macrocyclic lactams is shown in Scheme 2-2, in which Pei *et al.* discovered that 15-17 membered rings provided the best inhibitor properties against an enzyme involved in bacterial biosynthesis.[74]

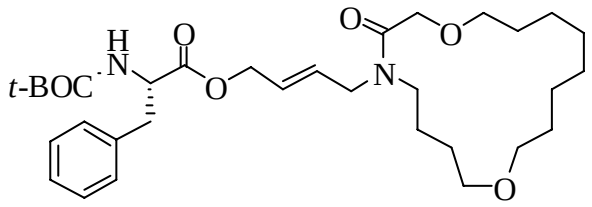


2-4 **2-5**

Scheme 2-2. Synthesis of a macrocyclic inhibitor against an enzyme found in bacteria

The examples above demonstrate the importance of macrocyclic lactams as scaffolds in drug development. As part of our continuing interest in cyclic peptidomimetics, we envisioned RCM as a means to generate macrocyclic scaffolds and cross-metathesis (CM) as a way to anchor amino acids to the scaffolds. Model system **2-6** is ideally suited for a CM reaction[1,33,78,79] because it has a dumb-bell shape with two

halves connected together by an *E*-alkene tether (Figure 2-2). We believed that Grubbs' well-defined second generation catalyst **1-4**, which has a high tolerance of functional groups, would be an ideal choice to facilitate this reaction.[80-82]



2-6

Figure 2-2. Phenylalanine on a macrocyclic lactam

In addition to examining RCM and CM as a synthetic approach toward peptidomimetics, we also wanted to test the reversibility of the CM reaction in this model system for potential use in dynamic combinatorial chemistry (DCC) of cyclic peptidomimetics.[83,84] We envisioned a macrocyclic scaffold possessing several anchoring points where pharmacophoric groups could be attached by a CM reaction, as shown in Figure 2-3. The CM reaction must be reversible in order for the pharmacophoric groups to attach and detach onto the scaffold in a dynamic equilibrium. Olefin metathesis is known to be reversible, but only Miller *et al*. have systematically examined the reactivity and selectivity of CM and the effects of remote functionality for potential use in DCC.[60] However, the work conducted by Miller[60] and other researchers[59,61] focused on the homodimerzation of their substrates. In contrast, our focus is on anchoring amino acid derivatives on molecular scaffolds. Dynamic combinatorial libraries (DCL) of cyclic peptidomimetics will be discussed further in Chapter 3. The main focal point of this chapter will be on the synthesis of model compound **2-6** by RCM and CM, followed by examination of the reversibility of the CM reaction.

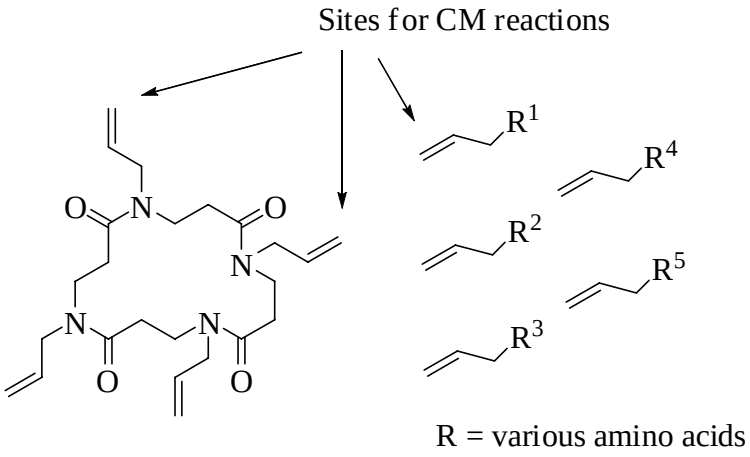
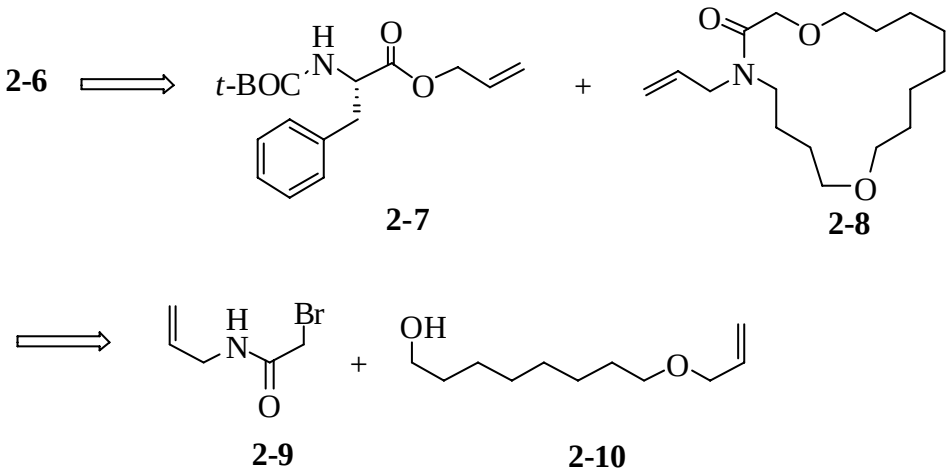


Figure 2-3. Macrocyclic lactam with anchors for CM with amino acids

In examining the viability of our approach to the synthesis of **2-6**, we decided that anchoring the amino acid to the nitrogen atom of a large-ring lactam would function well, as shown in Scheme 2-3. Compound **2-6** could be prepared from protected L-phenylalanine **2-7** bearing an allyl ester and allyl lactam **2-8**. Each of these halves of the molecule contains a terminal alkene to be used in the CM reaction. Precursor **2-8** was to be prepared by RCM and the alkene would later be removed by hydrogenolysis. Eventually we envisioned **2-8** as emanating from the S_N2 coupling of bromo-amide **2-9** and alcohol **2-10**.

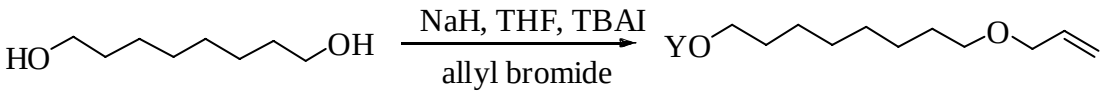


Scheme 2-3. Retrosynthethic analysis of **2-6**

2.2 Results and Discussion

2.2.1 Synthesis of Compound **2-6**

We began our synthesis with a Williamson etherification using commercially available 1,8-octanediol (**2-11**) and allyl bromide, as shown in Scheme 2-4. The desired allyl ether **2-10** was obtained in 70% yield, while the minor product, double Williamson ether **2-12**, was isolated in 25% yield. The use of tetrabutylammonium iodide (TBAI) was essential in obtaining high yields.[85]

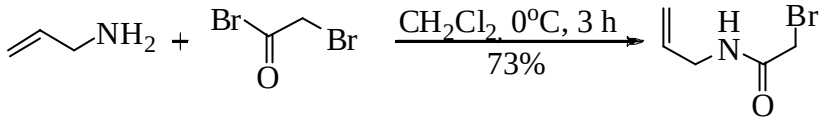


2-11

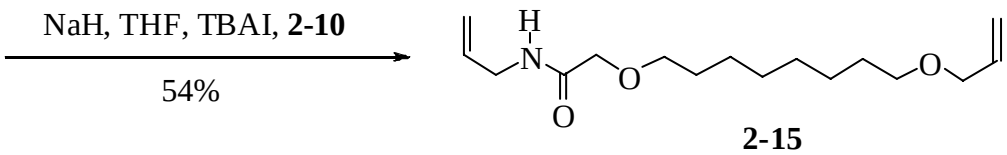
2-10: Y = H, 70%
2-12: Y = allyl, 25%

Scheme 2-4. Synthesis of **2-10** and **2-12**

N-Allyl-2-bromoacetamide (**2-9**) was next readily synthesized in 73% yield from allyl amine (**2-13**) and dibromide **2-14** followed by recrystallization in hexane/ether,[86] as shown in Scheme 2-3. Deprotonation of **2-10** with NaH, addition of TBAI, and nucleophilic substitution of allyl-bromoacetamide **2-9** gave a 54% yield of the terminal diene **2-15**.



2-13 **2-14** **2-9**

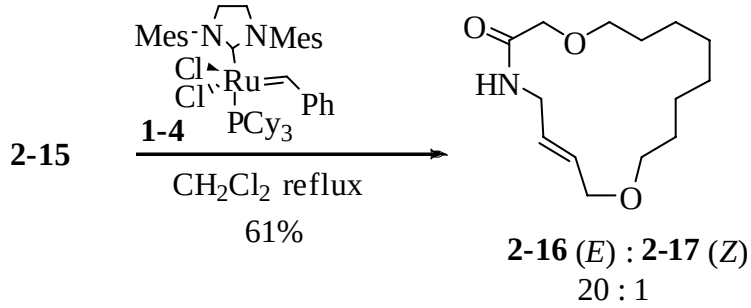


2-15

Scheme 2-5. Synthesis of diene **2-15**

Diene **2-15** was reacted with Grubbs' catalyst **1-4** under various conditions (Scheme 2-6 and Table 2-1) to give two 17-membered RCM lactams, **2-16** and **2-17**, as geometric isomers.[3] All reactions were refluxed in CH_2Cl_2 and monitored by thin layer chromatography (TLC). In every entry, **2-15** was never fully consumed. Moreover, data showed that refluxing for longer periods of time did not improve the yield of RCM products **2-16** and **2-17**.

The stereoselectivity of non-rigid macrocyclic alkenes formed from RCM is difficult to predict,[27] thus we were pleased with the excellent selectivity of our macrolactam. However, the next synthetic procedure required the hydrogenation of the alkene making steroselectivity a non issue. None the less, the *E*-isomer **2-16** was isolated as the major product, which was separated from *Z*-isomer **2-17** by chromatography over silica gel. For entries 6-8, the *cis*-isomer was not isolated due to small scale reactions.



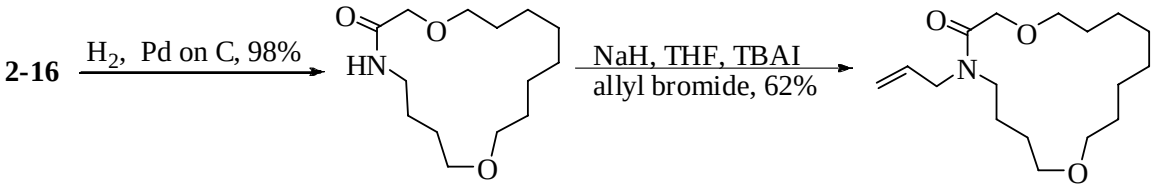
Scheme 2-6. RCM of **2-15**

After RCM, cycloalkenes **2-16** and **2-17** were hydrogenated using Pd on activated C (10% Pd) as the catalyst to give the corresponding saturated lactam **2-18** in high yields (Scheme 2-7). NMR spectra and TLC showed that it was pure enough for the next step without chromatography. Addition of NaH, TBAI, and nucleophilic substitution of allyl bromide, gave **2-8** in 62% yield. Two different conformations of **2-8**, in roughly equal

amounts, were present at ambient temperature and readily detected by ^{1}H NMR and ^{13}C NMR by the doubling of peaks.

Table 2-1. RCM to bbtain **2-16** and **2-17**

Entry	Conc (M)	**1-4** mol%	Time (h)	Yield %	E:Z **2-16:2-17**
1	0.001	10	25	59	93:7
2	0.0014	10	21	45	98:2
3	0.0016	10	24	46	92:8
4	0.0016	10	16	61	96:4
5	0.0018	8	19	60	93:7
6	0.003	14	3.5	48	NA
7	0.007	8	2	35	NA
8	0.013	5	20	18	NA

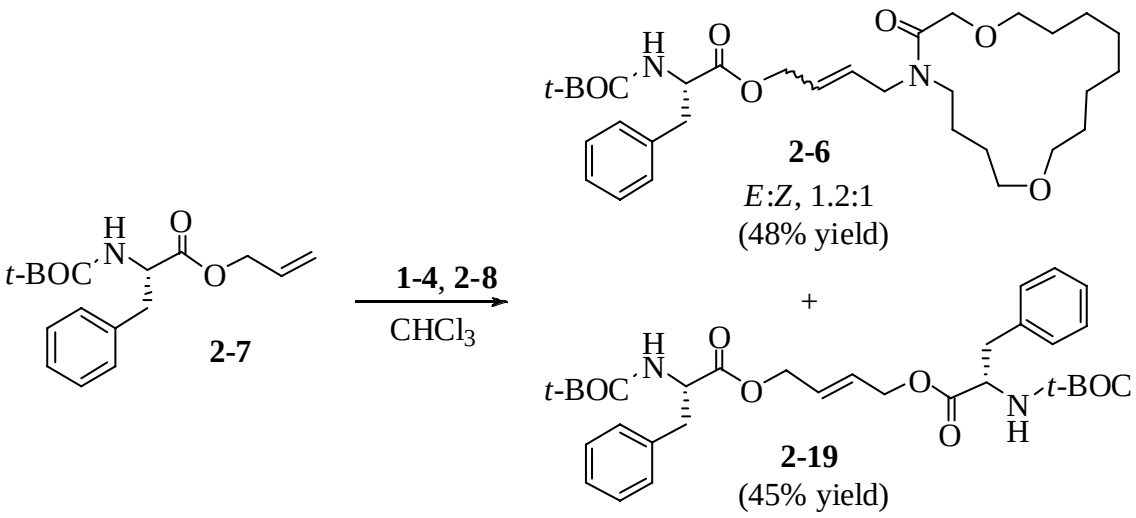


2-18 **2-8**

Scheme 2-7. Synthesis of lactam **2-8**

The next step involved the CM of the N-allyl lactam **2-8** with **2-7**, the allyl ester of *t*-BOC-phenylalanine (Scheme 2-8). To synthesize the amino acid derivative, commercially available *t*-BOC-L-phenylalanine was treated with 1,3-diisopropylcarbodiimide (DIC), hydroxybenzotriazole (HOBt) and allyl alcohol to give **2-7** in 95% yield. We were pleased that the CM of phenylalanine derivative **2-7** (2 equiv.) and *N*-allyl lactam **2-8** was observed with 5 mol% of catalyst **1-4**. The reaction was heated at 50 °C in $CHCl_3$ for 21 hours while continuously flushing out ethylene with argon to drive the reaction forward. Additional $CHCl_3$ was added as needed to keep the concentration to *ca.* 1M. After quenching the reaction with ethyl vinyl ether (EVE), purification gave the desired product **2-6** in 48% yield with an *E*:*Z* ratio of 1.2:1 and the

predominantly *trans* isomer of amino acid dimer **2-19** in 45%. Similar to the ^{13}C NMR of *N*-allyl lactam **2-8**, we also observed the doubling of peaks for **2-6**.



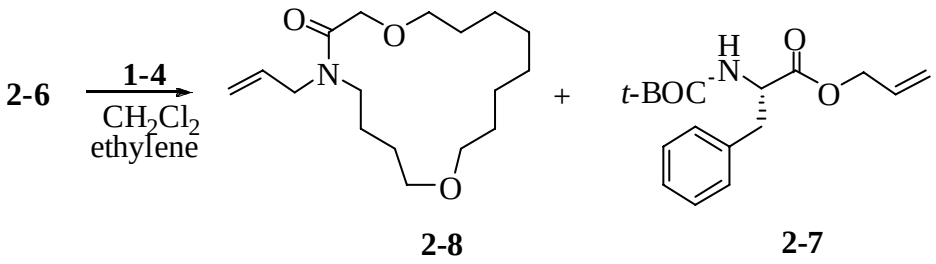
Scheme 2-8. CM reaction to obtain **2-6** and **2-19**

The moderate yields and lack of CM stereoselectivity of the heterodimer **2-6** were expected since both amino acid **2-6** and lactam **2-8** were considered Type I olefins with fast homodimerization reactivity,[33] as discussed in Chapter 1. Interestingly, the dimer of N-allyl lactam **2-8** was not observed. A 2:1 ratio of amino acid **2-6** and lactam **2-8** should result in a statistical heterodimer selectivity of 66%, which is slightly higher than the 52% selectivity we obtained. In regards to the stereoselectivity, the *trans* homodimers were predominantly formed, which is most likely due to secondary CM reactions to produce the more thermodynamically favored isomer.[33] However for heterodimer **2-6**, we believed that the bulky lactam may prevent *cis*/*trans* isomerization by secondary metathesis reactions, thus resulting in the lack of stereoselectivity.[33]

2.2.2 Examining the reversibility of the CM reaction in model **2-6**

Upon synthesizing compound **2-6**, we examined the reversibility of CM in this model system for potential use in dynamic combinatorial chemistry. It is known that CM

is reversible, and was tested in our system.[50,83,84] We were able to confirm that **2-6** can be converted back to allyl lactam **2-8** and amino acid derivative **2-7**, as shown in Scheme 2-9. Ethylene gas, used without purification, was admitted via a balloon to a stirred solution of compound **2-6**, catalyst **1-4** (2 mol%), and CH_2Cl_2. The solution was first maintained at room temperature overnight. TLC showed the formation of N-allyl lactam **2-8** and allyl ester **2-7**. In an attempt to drive the reaction further, the solution was refluxed in CH_2Cl_2 for 2.5 hours under an atmosphere of ethylene gas, then quenched with EVE upon cooling. Purification by chromatography gave 36% of *N*-allyl lactam **2-8** and 37% of allyl ester **2-7**. Both the amino acid dimer **2-19** (4%) and unreacted starting mater **2-6** (27%) were recovered as well. These studies demonstrated the potential of using allyl lactams and allyl amino acids as building blocks for dynamic combinatorial libraries of cyclic peptidomimetics.



2-8 **2-7**

Scheme 2-9. CM reaction with ethylene gas

2.3 Conclusion

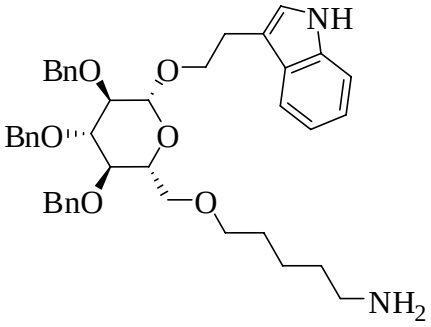
In summary, Grubbs' second generation ruthenium catalyst was used to couple the amino acid phenylalanine to a 17-membered lactam using CM in 48% yield with an *E*:*Z* ratio of 1.2:1. The CM product of two phenylalanine amino acids was isolated in 45% yield and found to be predominantly *trans*. A series of synthetic steps were needed to construct the 17-membered lactam, including the fundamental RCM reaction. The

reversibility of the cross metathesis of this macrocyclic system was demonstrated, which is essential to the development of a dynamic combinatorial library. Based on the results of our model study, the next goal was to generate a small library of cyclic peptidomimetics and determine how a template would shift the dynamic equilibrium.

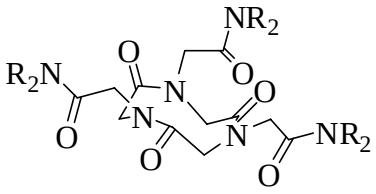
CHAPTER 3
OLEFIN METATHEIS OF AMINO ACID DERIVATIVES WITH CYCLIC SCAFFOLDS

3.1 Introduction

As discussed in Chapter 2, one of the approaches to the development of peptidomimetics is to attach biologically significant functional groups. An example of a scaffold used for drug development is shown in Figure 3-1, where amino acids are linked to a carbohydrate scaffold.[45] Another important type of scaffold is one that is peptide based. For example, glycine based scaffolds are of interest because it has been shown that they can potentially act as host molecules.[87]



Glucose-based nonpeptide scaffold



R = CH_2CH_2OBn

Glycine-based scaffold

Figure 3-1. Examples of scaffolds

By having terminal olefin moiety attached to peptide based scaffolds, there is potential to create a dynamic combinatorial library of peptidomimetics via olefin cross-metathesis (CM). Numerous studies have shown the advantages of dynamic combinatorial chemistry over traditional combinatorial chemistry. Employing olefin metathesis in dynamic combinatorial chemistry can efficiently lead to the synthesis and isolation of biologically significant peptidomimetics. There have been no reports in

literature where cyclic scaffolds and amino acid building blocks were used to create a dynamic library of peptidomimetics.

As part of our ongoing study directed toward the attachment of amino acid derivatives to a cyclic scaffold by olefin CM,[88] our research group is interested in studying the viability of reversible olefin CM for dynamic combinatorial chemistry of cyclic peptidomimetics. The constituents of our library are made by CM of amino acid precursors with a cyclic scaffold (Figure 3-2). Both the amino acid precursor and the cyclic molecule must possess a terminal alkene. We are examining the reactivity of various types of amino acid precursors and cyclic scaffolds. In addition, several templates are also being tested on the reaction to select for the best constituent in the library.

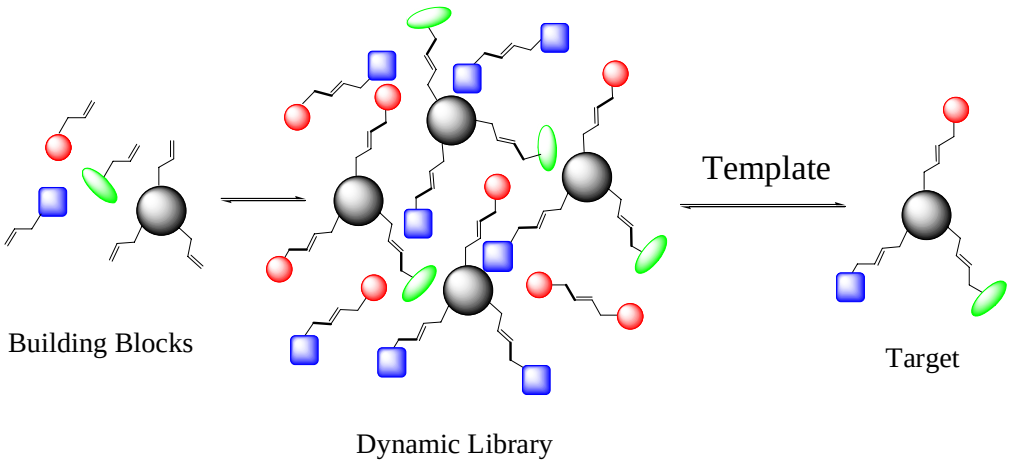
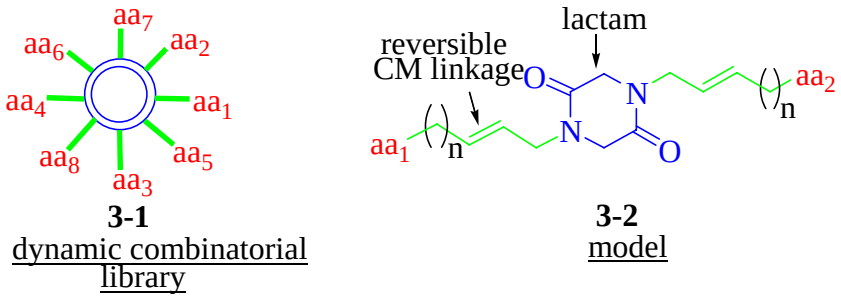


Figure 3-2. Dynamic combinatorial libraries of peptidomimetics

Amino acids can be combinatorially arranged around a glycine based lactam by a reversible CM reaction, as shown in Figure 3-3, to prepare a dynamic combinatorial library **3-1** on a cyclic scaffold. To examine the viability of the approach, model compound **3-2** was utilized in anchoring amino acid derivatives to the nitrogen atoms of a lactam.



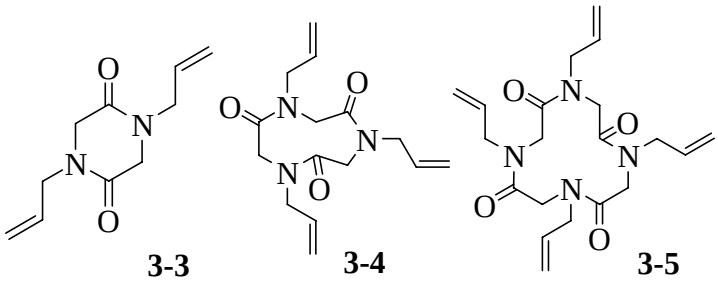
3-1
dynamic combinatorial library

3-2
model

Figure 3-3. Schematic of a dynamic combinatorial library and a model of amino acids linked to a cyclic scaffold by olefin CM

Miller and coworkers recently demonstrated the importance of remote functionality of olefin CM using Grubbs' first generation catalyst **1-2** for potential use in DCC.[60] Here we report the product yields and distributions of CM reactions of allyl and homoallyl ester amino acid derivatives with a rigid cyclic scaffold using Grubbs' second generation catalyst **1-4**.

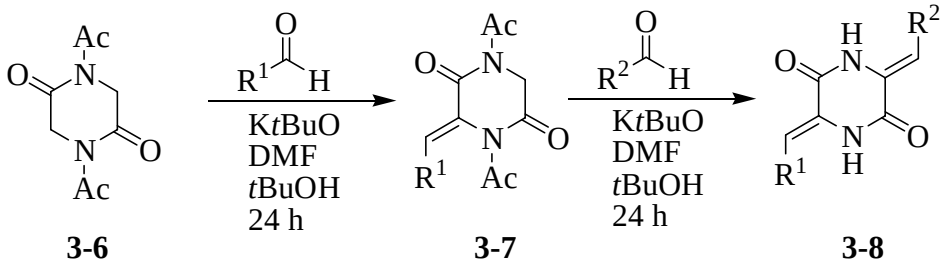
Several cyclic scaffolds are in consideration in these studies – dimer **3-3**, trimer **3-3**, and tetramer **3-5** (Figure 3-4). Dimer **3-3** is of interest because cyclic dipeptides possess important medicinal and biological characteristics.[40,89-91] In comparison to linear peptides, diketopiperazines are conformationally constrained and more stable against hydrolysis, which is critical in drug design.[92-94] In addition, the diketopiperize peptide derivatives could also be used as building blocks for the synthesis of larger or more complexed cyclic peptides.[91]



3-3 **3-4** **3-5**

Figure 3-4. Glycine-based dimer, trimer and tetramer scaffolds

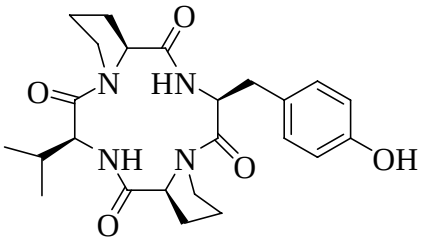
There has been great interest in combinatorial synthesis of cyclic dipeptide derivatives.[91,95] For example, Loughlin *et al*. demonstrated the solution-phase combinatorial synthesis and evaluation of piperazine-2,5-dione derivatives for cyctotoxic activities (Scheme 3-1).[95] They were able to discover important pharmacophoric groups based on their efficient combinatorial approach.



3-6 **3-7** **3-8**

Scheme 3-1. Combinatorial synthesis of piperazine-2,5-dione derivatives

We are also interested in synthesizing triallyl-*cyclo*-triglycine **3-4** to serve as the trimer scaffold. Similar to diketopiperazines, cyclic tripeptides possess a rigid conformation and display important pharmaceutical properties.[48] In addition to cyclic dipeptides and tripeptides, the ability to create a dynamic combinatorial library of molecules that mimic cyclic tetrapeptides would be important since they can also exhibit phytotoxic, cytotoxic,[96] and medicinal properties.[41] An example of a natural cyclic tetrapeptide is shown in Figure 3-5. Comparable to the structures of the dimer and trimer scaffolds, tetrallyl-cyclo-tetraglycine **3-5** can serve as a tetramer scaffold.



3-9

Cyclo(Proline-Valine-Proline-Tyrosine)
Tyrosinase inhibitor

Figure 3-5. Natural cyclic tetrapeptide

To generate a library of peptidomimetics, various amino acids possessing terminal olefin moiety can couple with these cyclic scaffolds by olefin CM. For example, the metathesis of two amino acid derivatives with *N*-allyl glycine scaffold **3-3** can lead to 3 homodimer and 3 heterodimer constituents (Figure 3-6). If considering *cis*/*trans* isomers, then there are 16 total possible products (Figure 3-7). Products from oligomerization of the dimer scaffold or olefin isomerization are excluded from these projected numbers.[97,98] The number of homodimers generated by metathesis is given by $N*(N+1)/2$ or $N*(N+1)$ including *E/Z* isomers, where *N* is the number of unequivalent amino acid derivatives in the pool. There is a great interest in synthesizing cyclic diketopiperazine peptide derivatives. However, these simple systems for use in dynamic combinatorial chemistry is not practical since at most, only two different amino acids could be attached to the dimer scaffold. The main interest in using the dimer scaffold in these experiments is to better understand the CM reactivity, yield, and stereochemistry of the expected products in a simple dynamic combinatorial system.

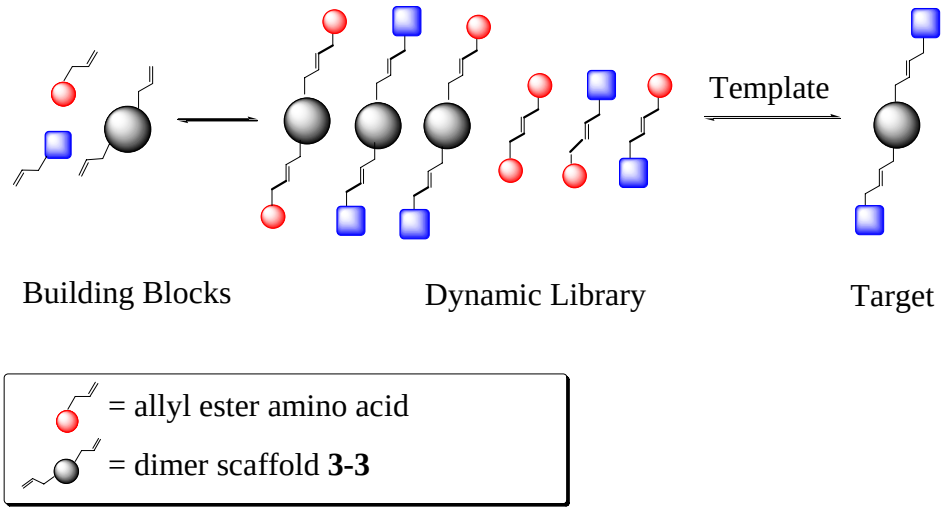


Figure 3-6. Small dynamic library of cyclic dipeptidomimetics

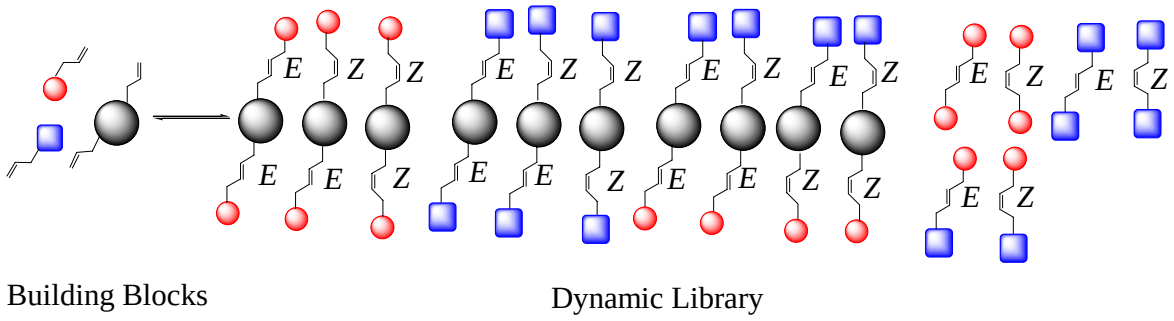


Figure 3-7. Dynamic library of cyclic dipeptidomimetics including *cis/trans* isomers

Employing the trimer scaffold **3-4** can lead to a larger library. If three amino acid derivatives react with the trimer scaffold in CM coupling, then the library will be composed of 10 unique cyclic molecules and 6 homodimers (Figure 3-8). This is excluding any olefin isomerization products or oligomerization of the cyclic scaffold. If stereoisomers are considered, then there are 48 isomers of cyclic molecules and 12 isomers of homodimers (Figure 3-9)

CM of tetramer scaffold **3-5** with four different amino acid derivatives would result in a larger library. There can potentially be 55 cyclic peptidomimetics (652 including *E*/*Z* isomers) and 10 homodimers (20 including *E*/*Z* isomers) (Figures 3-10 and 3-11).

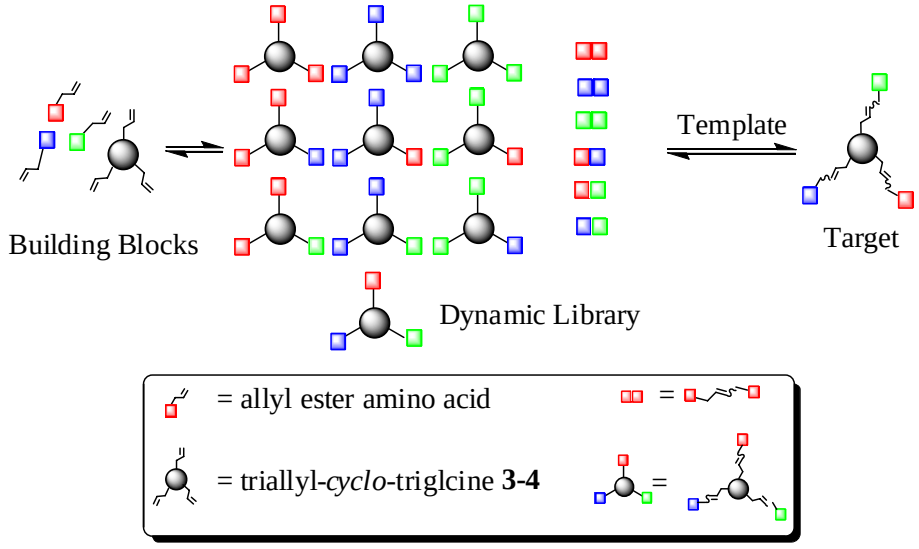


Figure 3-8. Dynamic library of cyclic tripeptidomimetics

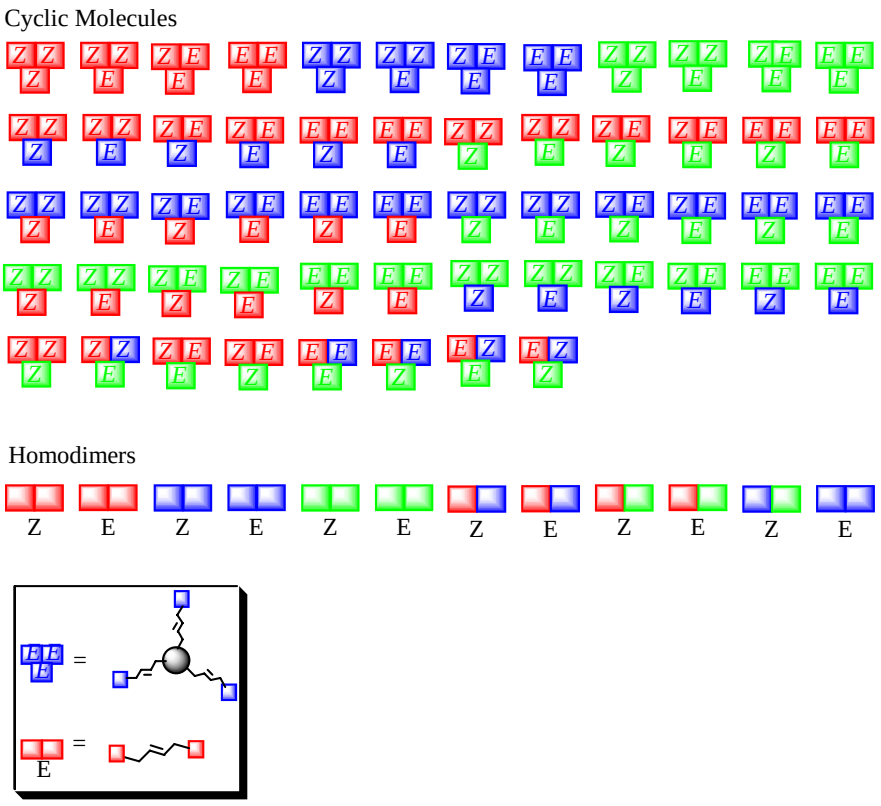


Figure 3-9. Dynamic library of cyclic tripeptidomimetics including *cis/trans* isomers

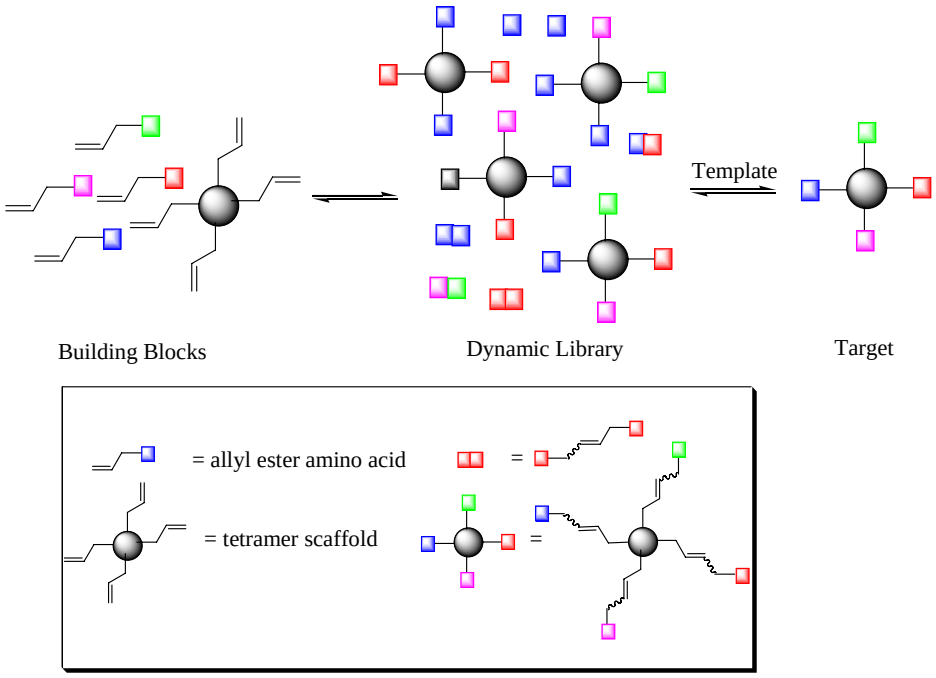


Figure 3-10. Dynamic library of cyclic tetrapeptidomimetics

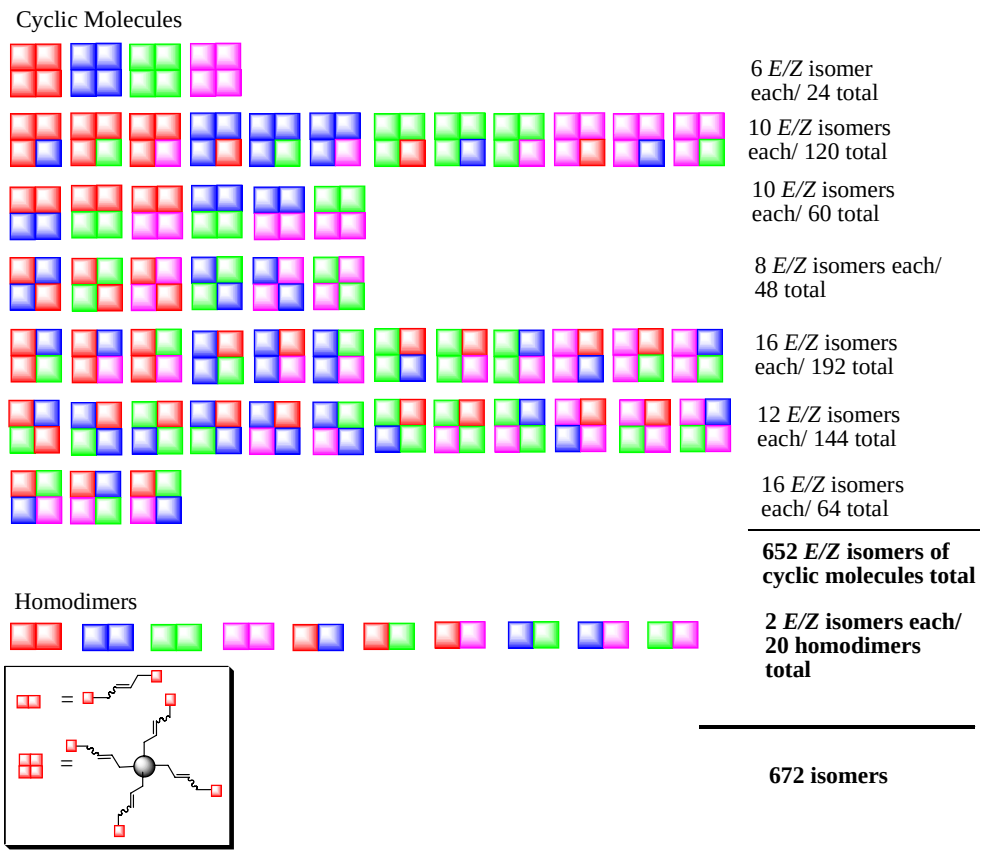


Figure 3-11. Dynamic library of cyclic tetrapeptidomimetics including *cis/trans* isomers

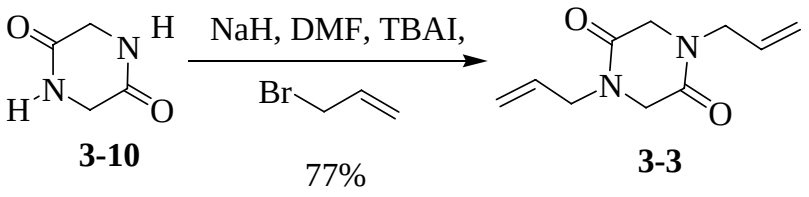
3.2 Results and Discussion

In order to study the viability of olefin metathesis for dynamic combinatorial chemistry, we first needed to synthesize the various monomers – the cyclic scaffolds and amino acid derivatives. It is then necessary to react the monomers with Grubbs' second generation catalyst to study the olefin CM selectivity and reactivity. The generation of a small library and template effects were also examined.

3.2.1 Synthesis of Cyclic Scaffolds

To obtain the N-allyl dimer scaffold **3-3**, NaH was added to a solution of commercially available glycine anhydride (**3-10**) and DMF (Scheme 3-2). Excess allyl bromide was added and the reaction was stirred at 70 $^{\circ}$C to give the desired product in 4 h. However, workup of the reaction proved to be cumbersome. Due to the polarity, some

of dimer **3-3** would remain in DMF during the aqueous workup even after numerous extractions with organic solvents. The best method we found to obtain good yields was to quench the reaction with H_2O, then remove the DMF and water *in vacuo* with heat. The residue was redissolved in EtOAc, leaving behind the sodium salts which were filtered off. Concentration *in vacuo* and purification by chromatography gave the desire product as a white solid in good yields.



3-10 **3-3**

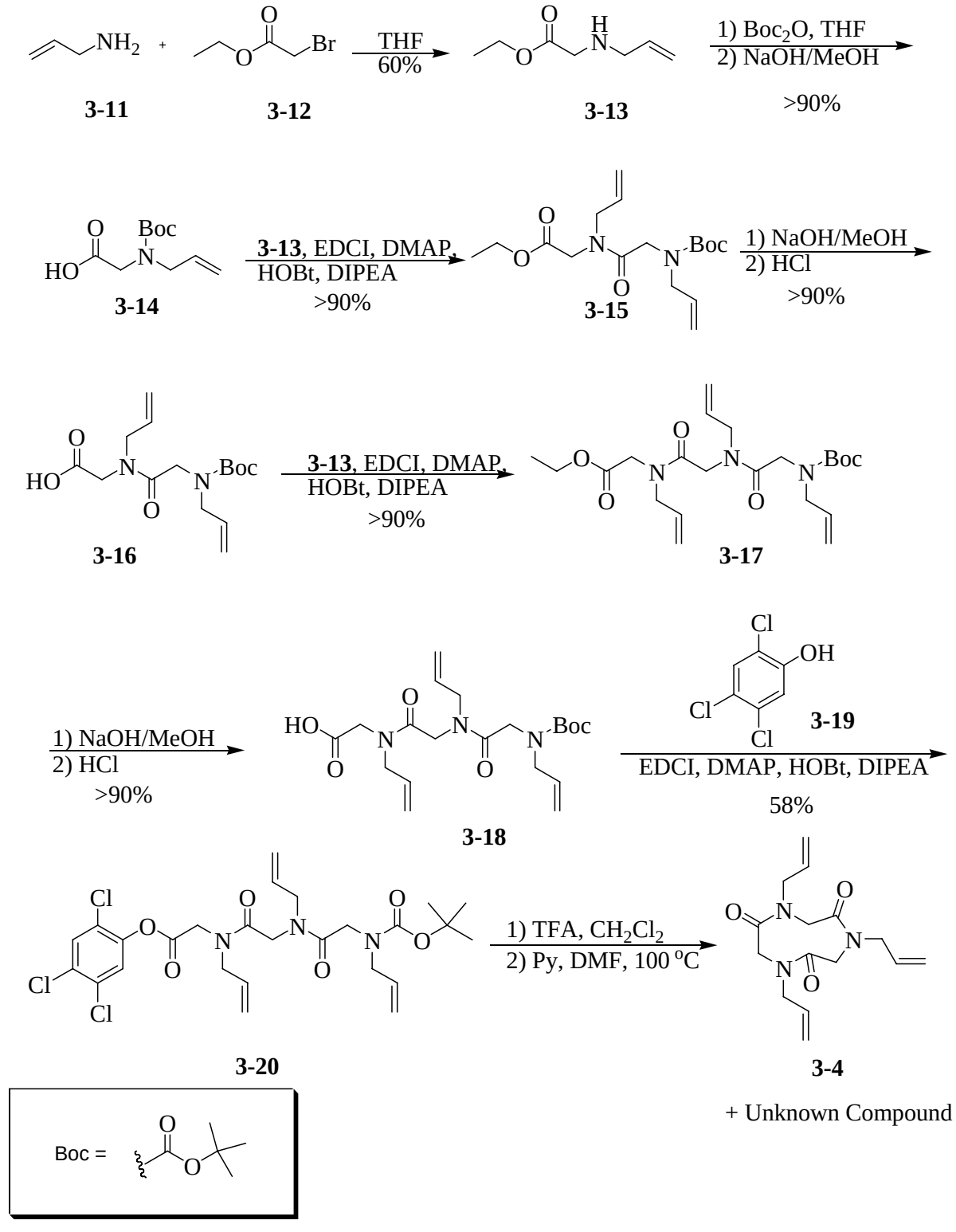
Scheme 3-2. Synthesis of dimer scaffold

Triallyl-*cyclo*-triglycine **3-4** was prepared following literature procedures,[47,87] with modifications as described in the experimental section (Scheme 3-3). Procedures consisted mainly of hydrolysis and typical amino acid coupling. Liskamp's group used Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) as the coupling reagent.[47] We chose N-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDCI) as our coupling reagent simply because it was less expensive and we were able to obtain high yields. In addition to EDCI, 4-dimethylaminopyridine (DMAP), diisopropylethylamine (DIPEA, Hunig's base), and hydroxybenzotriazole (HOBt), were used with the coupling reagent. All of the above hydrolysis and condensation reactions gave high yields comparable with literature, and typically did not require purification by column chromatography.

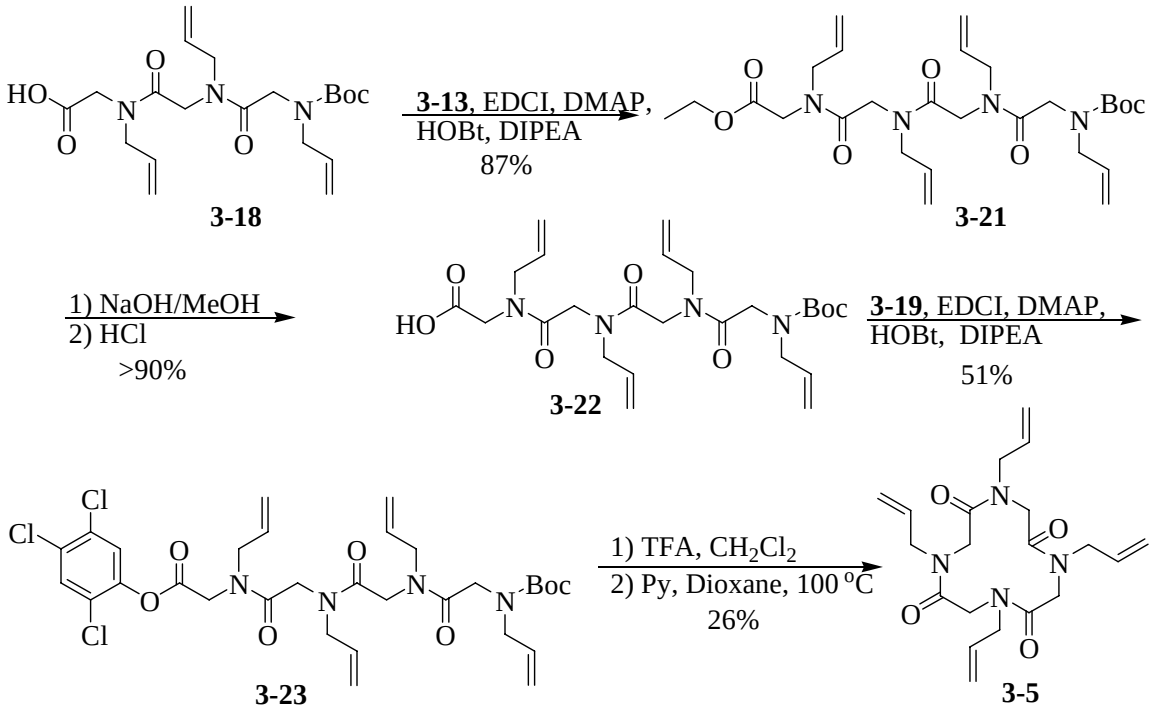
Trichlorophenol **3-19** was coupled to acid **3-16** to afford compound **3-20**.[99] After removing the t-Boc protecting group from ester **3-20** with trifluoroacetic acid (TFA), we

attempted to cyclize the compound. The reaction was run in DMF under very dilute conditions (4 mM) to preferentially form the cyclic trimer rather than the cyclic hexamer. Closure to form a nine member ring is known to be difficult.[28] Hioki *et al.* reported a 11% yield for the cyclic trimer, with the cyclic hexamer as the major product.[87] Upon purification by column chromatography, we isolated a white solid appearing to be either the trimer or the hexamer, according to ^{1}H NMR and ^{13}C NMR. High resolution mass spectroscopy (HRMS) indicated the presence of trimer **3-4** and an unknown compound. A pure sample of trimer could not be isolated even after numerous recrystallizations in various solvent systems, or by column chromatography.

Tetramer scaffold **3-5** was synthesized through simple hydrolysis and condensation starting from acid **3-18** (Scheme 3-4). The t-Boc protecting group of trichloro ester **3-23,** was removed by TFA. Using similar procedures for the cyclization of the trimer, the ester was heated for 24 h in dioxane (4 mM) and pyridine (1.1 eq). We observed two new distinct spots on TLC with R_f 0.37 and R_f 0.24 in EtOAc. Both spots were isolated as a white solid by column chromatography. Based on ^{1}H NMR, ^{13}C NMR, HRMS, and combustion analysis, the more polar compound with R_f 0.24 was tetramer **3-5** (26% yield). HRMS, ^{1}H NMR, and ^{13}C NMR analysis of the compound with R_f 0.37 indicate an unsymmetrical conformation of tetramer **3-5** (11%). Identical results were obtained using DMF, but this is a less desirable solvent because of the difficulty of removing DMF during the workup.



Scheme 3-3. Synthesis of trimer scaffold

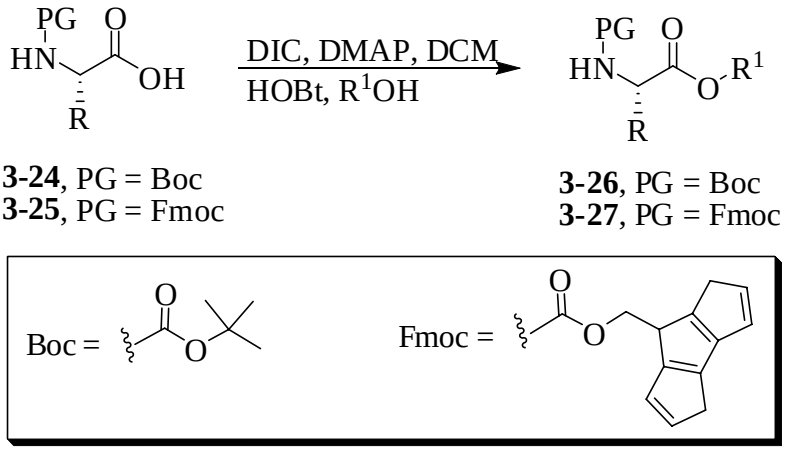


Scheme 3-4. Synthesis of tetramer scaffold

3.2.2 Synthesis of Amino Acid Derivatives

Different amino acid derivatives were synthesized to determine the effects of protecting groups, side chains, and alkene moieties in olefin cross-metathesis. McNaugton *et al*. had examined the effect of remote functionality but with allyl and homoallylamides.[60] Herein, we investigated the allyl and homoallyl olefin moiety of the carboxylate end of the amino acid.

A series of amino acid derivatives were synthesized by coupling t-Boc or Fmoc protected amino acids with allyl alcohol or 3-buten-1-ol using DIC, DMAP, and HOBt (Scheme 3-5). The starting material was typically consumed within 20 minutes as indicated on TLC. The urea byproduct was filtered off and purification by column chromatography gave the amino acid products in high yields (Table 3-1 and Figure 3-12). MeOH was used as the solvent to determine the optical rotation of the samples.



Scheme 3-5. Synthesis of amino acid derivatives

Table 3-1. Yields, melting points and optical rotations of amino acid derivatives

Entry	*N*-PG-AA	R^1	Product	Yield (%)	mp (°C)	$[\alpha]_D$(°) (°C, *c*)
1	Boc-Phe **3-24a**	Allyl	**3-26a**[1]	>95	71-72	-8.05 (25, *c* = 1.10)
2	Boc-Ala **3-24b**	Allyl	**3-26b**	>95	oil	-35.0 (25, *c* = 1.04)
3	Boc-Pro **3-24c**	Allyl	**3-26c**	95	oil	-70.9 (25, *c* = 1.00)
4	Boc-Met **3-24d**	Allyl	**3-26d**	>95	oil	-32.4 (25, *c* = 1.04)
5	Boc-Phe **3-24e**	Homoallyl	**3-26e**	90	79-80.5	-9.01 (25, *c* = 1.00)
6	Boc-Ala **3-24f**	Homoallyl	**3-26f**	98	oil	-45.7 (25, *c* = 1.11)
7	Boc-Pro **3-24g**	Homolallyl	**3-26g**	89	oil	-72.3 (25, *c* = 1.24)
8	Boc-Met **3-24h**	Homoallyl	**3-26h**	88	oil	-23.8 (25, *c* = 1.10)
9	Boc-Leu **3-24i**	Homoallyl	**3-26i**	86	oil	-39.2 (25, *c* = 1.42)
10	Fmoc-Phe **3-35a**	Homoallyl	**3-27a**	>95	52-54	-20.0 (25, *c* = 1.06)
11	Fmoc-Pro **3-25b**	Homoallyl	**3-27b**	92	oil	-49.4 (25, c = 1.25)
12	Fmoc-Gly **3-26c**	Homoallyl	**3-27c**	85	78.5-80	N/A

[1] Allyl Boc-Phe was first discussed in chapter 2 and referred as compound **2-7**. For clarity and simplicity in the numbering of our libraries, the compound will be labeled as **3-26a**.

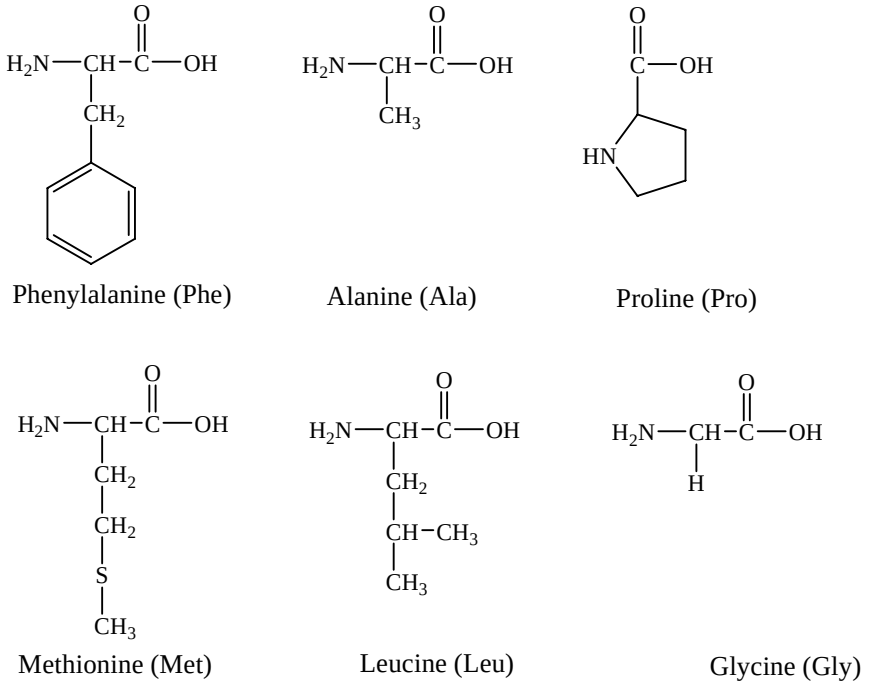


Figure 3-12. Amino acids used in the library.

As shown in Table 3-2, HOBt was required to prevent racemization of the chiral center. The ^{1}H NMR and optical rotation data of allyl ester phenylalanine **3-26a**[100] and alanine **3-26b**[101] were similar to those reported in literature. Full characterization of the other amino acid derivatives is found in Chapter 6, experimental section.

Table 3-2. Yields, melting points, and optical rotations of amino acid derivatives without use of HOBt during synthesis

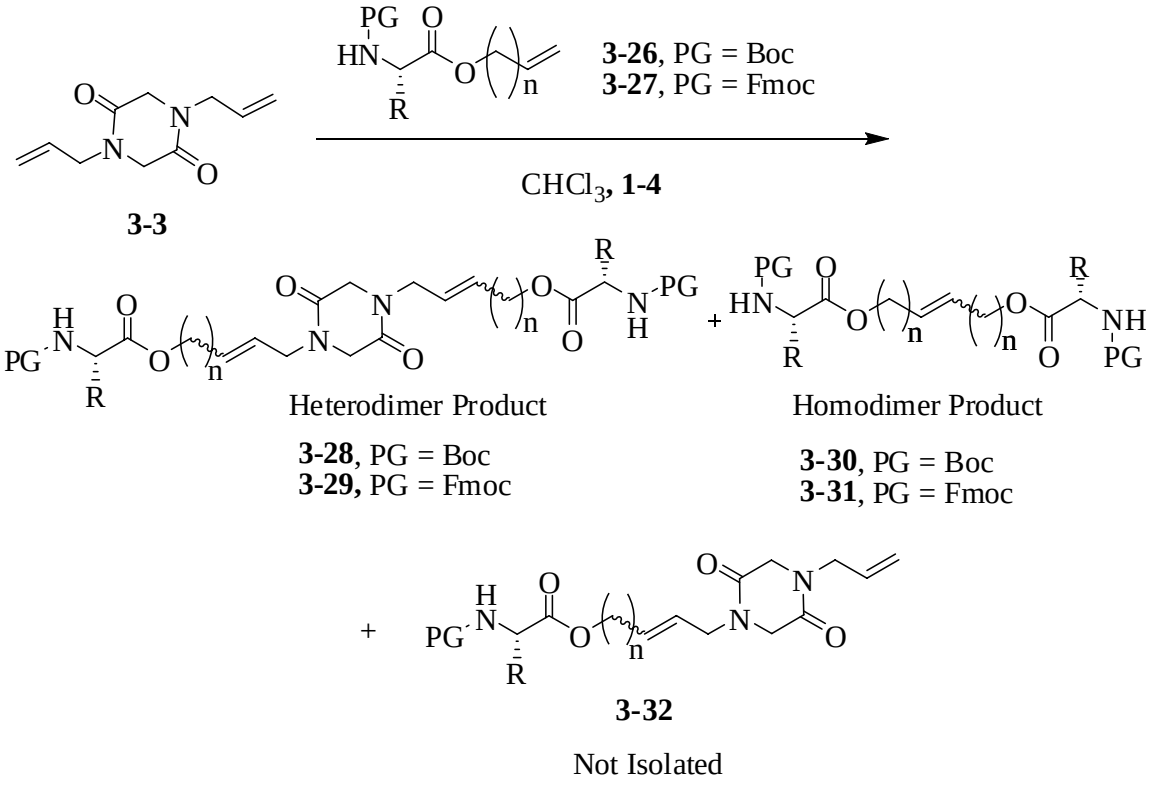
Entry	*N*-PG-AA	R^1	Product	Yield (%)	mp (°C)	$[\alpha]_D$(°) (°C, *c*)
1	Boc-Phe	Allyl	**3-26a**	87	70-71	-2.31 (25, *c* = 1.00)
2	Boc-Ala	Allyl	**3-26b**	88	oil	-13.6 (25, *c* = 1.08)
3	Boc-Phe	Homoallyl	**3-26e**	56	78-80	-2.20 (25, *c* = 1.00)
4	Boc-Ala	Homoallyl	**3-26f**	80	oil	-33.4 (25, *c* = 1.00)

3.2.3 CM reactivity of Dimer with One Amino Acid

The CM reactivity of dimer scaffold **3-3** with amino acid derivatives possessing different protecting groups and olefin moiety was examined. Dimer scaffold **3-3** was

allowed to react with an amino acid derivative (5 eq.) using 10 mol% Grubbs' second generation catalyst **1-4** (Scheme 3-6). The reaction was stirred at reflux in $CHCl_3$ for 10 h while flushing the headspace with argon to remove evolved ethylene. The reaction was quenched with EVE and purification by column chromatography gave the desired products in moderate yield (Table 3-3).

We attempted to isolate compound **3-32**, the CM product of one amino acid derivative with dimer scaffold **3-3**, by column chromatography. Separation and purification of **3-32** from other byproducts was unsuccessful, but the mass spectrometer data did indicate the presence of compound **3-32**. The low yields of **3-32**were expected due to excess amount of amino acid derivatives which would favor the homodimer or heterodimer products. In addition, these CM reactions were run on a small scale.



Scheme 3-6. Olefin CM of dimer scaffold with an amino acid derivative

Table 3-3. Yields of heterodimers and homodimers

Entry	Starting Material	*N*-PG-AA	R^1, n	Hetero-dimer	Yield %	Homo-dimer	Yield %
1	**3-26a**	Boc-Phe	Allyl, n=1	**3-28a**	37	**3-30a**	38
2	**3-26b**	Boc-Ala	Allyl, n=1	**3-28b**	40	**3-30b**	42
3	**3-26c**	Boc-Pro	Allyl, n=1	**3-28c**	42	**3-30c**	31
4	**3-26d**	Boc-Met	Allyl, n=1	**3-28d**	0	**3-30d**	0
5	**3-26e**	Boc-Phe	Homoallyl, n=2	**3-28e**	44	**3-30e**	50
6	**3-26f**	Boc-Ala	Homoallyl, n=2	**3-28f**	39	**3-30f**	66
7	**3-26g**	Boc-Pro	Homoallyl, n=2	**3-28g**	45	**3-30g**	55
8	**3-26h**	Boc-Met	Homoallyl, n=2	**3-28h**	0	**3-30h**	14
9	**3-26i**	Boc-Leu	Homoallyl, n=2	**3-28i**	30	**3-30i**	59
10	**3-27a**	Fmoc-Phe	Homoallyl, n=2	**3-29a**	42	**3-31a**	48
11	**3-27b**	Fmoc-Pro	Homoallyl, n=2	**3-29b**	46	**3-31b**	52
12	**3-27c**	Fmoc-Gly	Homoallyl, n=2	**3-29c**	30	**3-31c**	44

[1] Isolated yields except **3-31b**, which is based on NMR

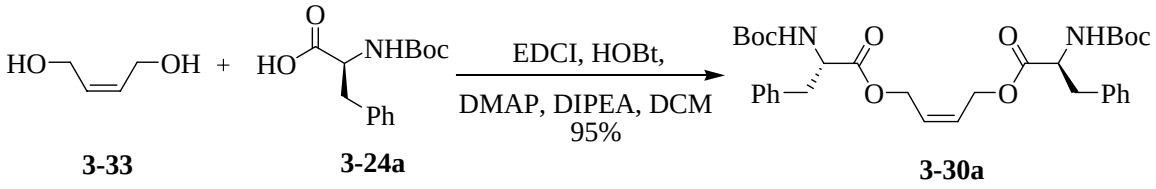
Similarly, the heterodimer products **3-28** and **3-29** were difficult to purify by column chromatography due to the polarity of the compound. Based on TLC, these compounds have R_f values similar to dimer **3-3** and compound **3-32.** Fortunately, we were able to isolate enough of the heterodimer product for characterization. For all of the CM reactions, the amino acid starting material was not fully consumed. The homodimer products had R_f values close to the amino acid starting material, but we were able to obtain pure samples by column chromatography. Homodimer products **3-30a**, **3-30b**, **3-30e**, **3-31a**, and **3-31c** were isolated as a solid.

The yields for the heterodimer products **3-28** and **3-29** were comparable to each other, whether an allyl or homoallyl olefin moiety was attached to the amino acid. There was also little difference between the yields of Fmoc protected **3-29a** and Boc protected **3-28a** and **3-28e**. However, the yields improved for the homodimerized products **3-30 e-h** and **3-31 a-b,** which possess the homoallyl olefin chain, in comparison to homodimerized products **3-31 a-d** with the allyl chain. A possible reason for the higher yield is that the ruthenium catalyst is less sterically hindered by the amino acid moiety and has better access to the longer chain terminal olefin.[26] Again we saw little differences between the Fmoc **3-31 a-b** and Boc protected **3-30e and 3-30g**. In all cases, CM of methionine derivatives resulted in zero or low yields. Analysis by TLC showed that mostly starting material **3-26d** and dimer **3-3** were present even after 2 days of reflux.[102] In contrast to McNaughton's work, our yields were overall higher by having the olefin moiety attached to the ester and the amide protected with Boc or Fmoc.

We examined the ^{1}H and ^{13}C NMR of the crude reaction mixture to determine the *cis*/*trans* ratio of homodimerized products **3-30** and **3-31**. The chemical shift of the *cis* isomers was expected to be further downfield than the *trans* isomers. However, the large number of library constituents in the crude reaction mixture made the NMR data complex. Therefore, we isolated the homodimerized products by column chromatography. Based on the NMR spectra, most of the isolated compounds appeared to a pure isomer, rather than a mixture of *cis* and *trans* isomers. We expected them to be mostly *trans* based on Grubb's studies.[33] However, experiments conducted by McNaugton *et al*. showed a 3:1 ratio of *cis*/*trans* but with the utilization of Grubbs first generation catalyst **1-2**.[60]

To ensure we properly assigned the *trans*/*cis* ratio of homodimer products, we examined the satellites of the alkene protons using a 500 MHz spectrometer with deuterated acetone as the solvent. These weak satellites were formed from protons attached directly to the ^{13}C (1% natural abundance), rather than protons attached to the more abundant ^{12}C isotope.[103] The satellites were located 80 Hz to the right and left side of the of the stronger proton signal. We expected the alkene protons of the *trans* isomers have a larger coupling constant (J = 15-17 Hz) than the *cis* isomers (J =9-11 Hz).[104]

We independently synthesized two authentic homodimers, where the stereochemistry was known, to confirm the predicted J coupling values of the weak satellites. We first synthesized the *cis* allyl homodimer **3-30a** by (*Z*)-2-butene-1,4-diol (**3-33**) with 3 equivalents of *N*-(tert-butoxycarbonyl)-phenylalanine (**3-24a**), DMAP, HOBt, DIPEA, and EDCI in 95% yield (Scheme 3-7). Homodimer **3-30a** was also synthesized using DIC as the coupling agent. However, removal of the urea byproduct was cumbersome, and the yield was 88%. NMR analysis of the weak satellites of the *cis* alkene protons indicated a pattern of a doublet of a triplet with J = 11 and 6 Hz (Figure 3-13).



3-33 **3-24a** **3-30a**

Scheme 3-7. Synthesis of *cis* homodimer **3-30a**

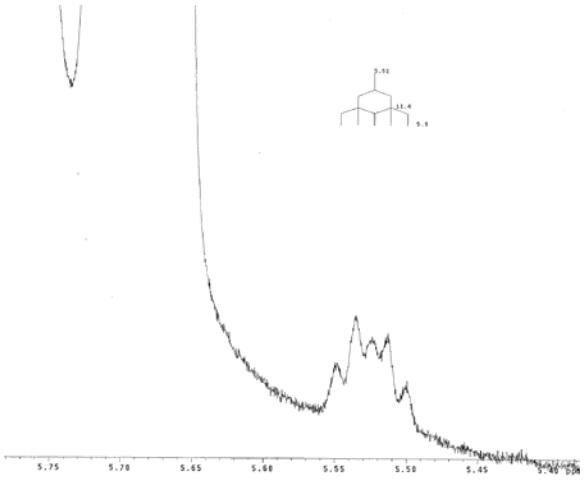
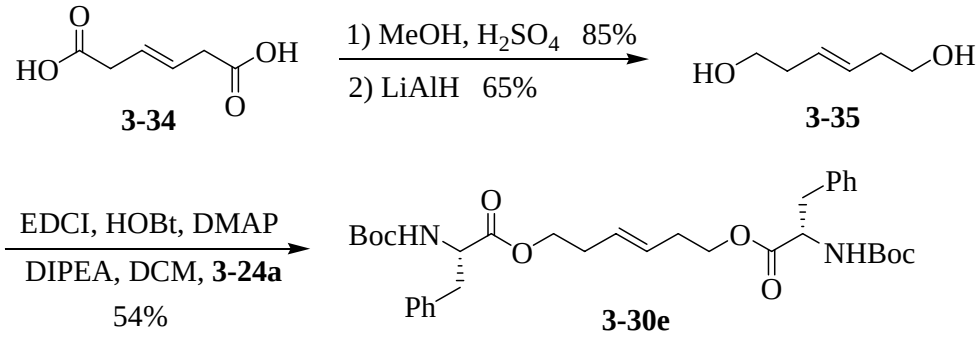


Figure 3-13. Satellites of the alkene protons from *cis* homodimer **3-30a**

We also independently synthesized the *trans* homoallyl dimer product by employing the same method as above, but starting from the less expensive *trans*-3-hexenedioic acid **3-34** rather than diol **3-35** (Scheme 3-8). The acid was easily reduced to the diol by LiAlH,[60] followed by amino acid coupling with Boc-protected L-phenylalanine **3-24a** to obtain the *trans* homodimer product **3-30e**. NMR analysis of *trans* **3-30e** and the weak satellites of the alkene protons indicated a doublet of a triplet pattern with J = 16 and 7 Hz (Figure 3-14).



Scheme 3-8. Synthesis of *trans* homodimer **3-30e**

NMR analysis of our homodimer samples from the CM reactions indicated the presence of *trans* isomers, as determined by the J coupling of the weak satellites. We observed a doublet of a triplet pattern with J = 16 and 7 Hz.

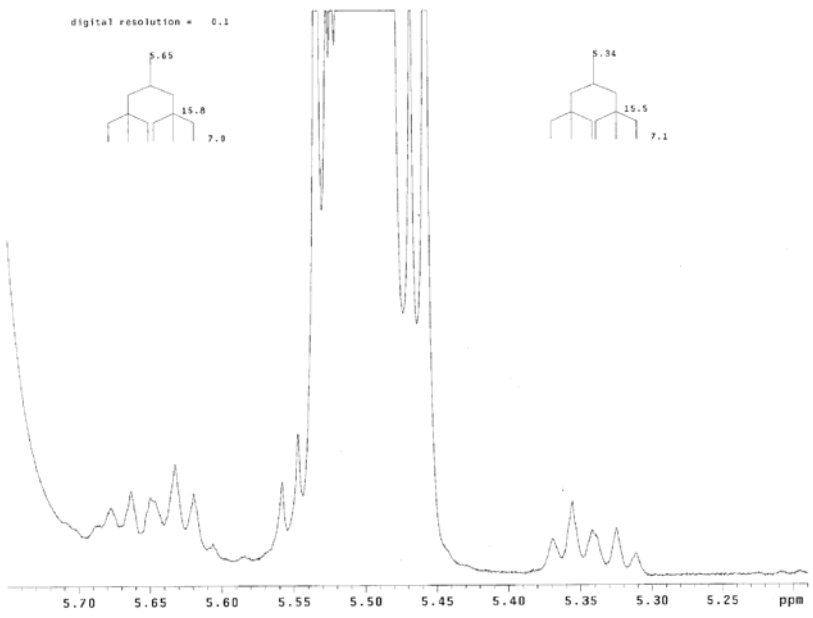


Figure 3-14. Satellites of the alkene protons from *trans* homodimer **3-30e**

3.2.4 Generation of Small Libraries and Template Effects

After examining the CM reactivity of the amino acid derivatives with the dimer scaffold, we were interested in generating a small library and examining the template effects. As a preliminary study, dimer **3-3** was allowed to react with 2 or 3 different amino acid derivatives (2-3 eq) in a cross-coupling reaction at room temperature or at reflux using 10-18 mol% of catalyst **1-4**. See Scheme 3-9 for a representative reaction. For this particular example, in which two different amino acids were used, we would expect to see three different homodimers and heterodimers, and possibly the mono-coupled products (Table 3-4). The resulting equilibrium mixture was frozen upon addition of EVE, which inactivated the catalyst. The catalyst was removed by washing with water soluble tris(hydroxymethyl)phosphine [THP, $P(CH_2OH)_3$][105] or through column chromatography. Isolation of each CM product by column chromatography was

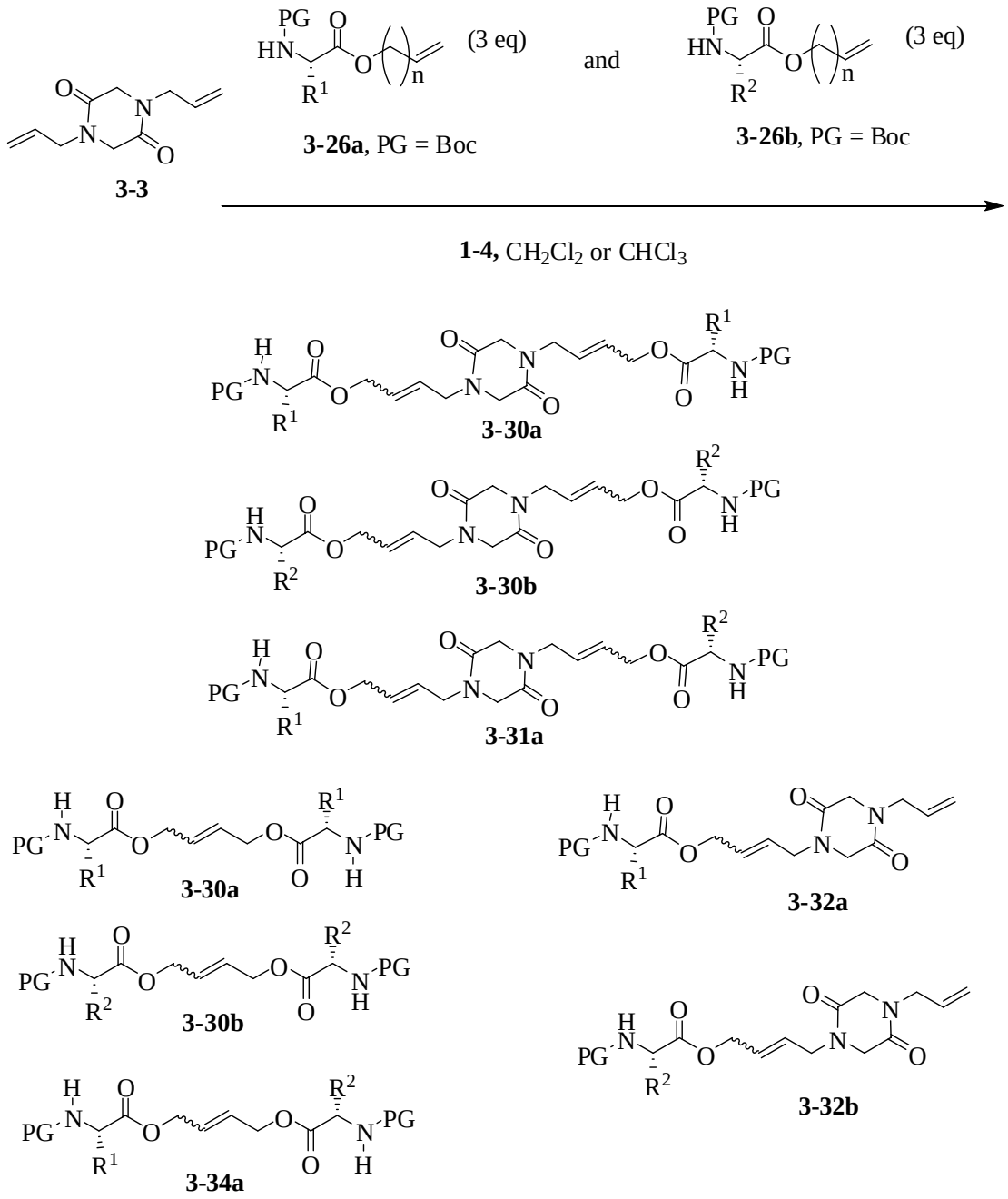
not feasible due to the number of possible CM products as well as any unreactive starting materials. Therefore, the samples were analyzed by HPLC.

Several groups have studied lithium salts and their effect on the conformation of peptides.[106,107] Sanders' group used hydrazone chemistry to prepare a dynamic combinatorial library of cyclic peptidomimetics.[58] They observed the formation of one major 42-membered macrocyclic compound upon addition of lithium salts. This is a surprising result since Li^+ is a small cation that wouldn't be expected to coordinate with a large cyclic compound. In our system, the heterodimer product has a linear configuration where the amino acid derivatives are separated by the cyclic scaffold. Because of this configuration, we would not expect a small ion to influence the dynamic equilibrium very much. Nevertheless, we were interested in knowing the effects, if any at all, of lithium salts on our small dynamic library. Several reaction conditions were examined to determine the effects of the lithium ion template (Table 3-5).

In experiments 1 and 2, the reaction mixture was refluxed for 11 hours without the addition of a template (Table 3-5). These reactions were compared to experiment 3, in which the dimer, amino acid derivatives, and catalyst were refluxed for 3 hours followed by the addition of the $LiClO_4$. The reaction mixture was then refluxed for another 8 hr. Based on HPLC data, there were no significant changes when the template was added to the reaction.

We then examined the influence of a proline derivative, which possesses a rigid structure compared to other amino acids. For experiments 3-7, the reaction was refluxed in its respected solvent as listed in Table 3-5. There were some changes to the peak patterns and area % of the HPLC traces of samples with and without $LiClO_4$. However,

there was no major increase in concentration of one or two peaks after the addition of $LiClO_4$ to conclude that the template influenced the equilibrium in a significant capacity.



Scheme 3-9. CM of dimer scaffold with two amino acid derivatives

Table 3-4. Expected products from CM reaction of dimer scaffold and two or three amino acid derivatives

Entry	Starting Material	Possible Products		
		Heterodimer	Mono-coupled	Homodimer
1	Dimer **3-3** Boc-Allyl Phe **3-26a** Boc-Allyl Ala **3-26b**	**3-28a** (Phe-D-Phe) **3-28b** (Ala-D-Ala) **3-33a** (Phe-D-Ala)	**3-32a** (D-Phe) **3-32b** (D-Ala)	**3-30a** (Phe-Phe) **3-30b** (Ala-Ala) **3-34a** (Phe-Ala)
2	Dimer **3-3** Boc-Homoallyl Phe **3-26e** Boc-Homoallyl Pro **3-26c**	**3-28e** (Phe-D-Phe) **3-28g** (Pro-D-Pro) **3-33b** (Phe-D-Pro)	**3-32c** (D-Phe) **3-32d** (D-Pro)	**3-30e** (Phe-Phe) **3-30g** (Pro-Pro) **3-34b** (Phe-Pro)
3	Dimer **3-3** Fmoc-Homoallyl Phe **3-27a** Fmoc-Homoallyl Pro **3-27b** Fmoc-Homoallyl Gly **3-27c**	**3-29a** (Phe-D-Phe) **3-29b** (Pro-D-Pro) **3-33c** (Phe-D-Pro) **3-33d** (Phe-D-Gly) **3-33e** (Gly-D-Pro)	**3-32e** (D-Phe) **3-32f** (D-Pro) **3-32c** (D-Gly)	**3-31a** (Phe-Phe) **3-31b** (Pro-Pro) **3-31c** (Gly-Gly) **3-34c** (Phe-Pro) **3-34d** (Phe-Gly) **3-34e** (Pro-Gly)

In experiment 8, the system was analyzed using Fmoc amino acid derivatives, which was also easier to observe by HPLC because of the aromatic rings. Grubbs' catalyst (13 mol%) was added to a solution of the dimer **3-3**, Fmoc-L-Phe **3-27a**, Fmoc-L-Pro **3-27b**, Fmoc Gly **3-27c**, and $CHCl_3$ at r.t. and stirred for 24 h to ensure equilibration (Table 3-5). LiCl (3 eq) was added and the reaction stirred for 1 h, followed by another addition of Grubbs (7 mol%). After stirring at room temperature for 21 h, additional LiCl (4 eq), Grubbs (7 mol%), and $CHCl_3$ (0.5 mL) were added. The reaction was maintained for another 23 h. In order to observe the influence of heat, Grubbs (4%) and $CHCl_3$ (1 mL) were added and reaction heated at 40 °C for 23 h. Aliquots of the reaction mixture were taken at numerous time points to monitor the changes by HPLC.

Similar to previous experiments, minor changes were observed but there was no dramatic shift in the equilibrium.

Table 3-5. CM conditions with and without lithium template

Experiment	Starting Material (equivalent)	Solvent (Molarity)	Catalyst **1-4** (mol-%)	Template (equivalent)
1	Dimer **3-3** (1 eq) Boc-Allyl Phe **3-26a** (2 eq) Boc-Allyl Ala **3-26b** (2 eq)	$CHCl_3$ (0.5 M)	10%	None
2	Dimer **3-3** (1 eq) Boc-Allyl Phe **3-26a** (3 eq) Boc-Allyl Ala **3-26b** (3 eq)	$CHCl_3$ (0.5 M)	10%	None
3	Dimer **3-3** (1 eq) Boc-Allyl Phe **3-26a** (3 eq) Boc-Allyl Ala **3-26b**(3 eq)	$CHCl_3$ (0.5 M)	10%	$LiClO_4$ (1 eq)
4	Dimer **3-3** (1 eq) Boc-Allyl Phe **3-26a** (2 eq) Boc-Allyl Pro **3-26g**(2 eq)	CH_2Cl_2 (0.7 M)	10%	$LiClO_4$ (1.4 eq)
5	Dimer **3-3** (1 eq) Boc-Homoallyl Phe **3-26e** (2 eq) Boc-Homoallyl Pro **3-26g** (2 eq)	$CHCl_3$ (0.4 M)	16%	None
6	Dimer **3-3** (1 eq) Boc-Homoallyl Phe **3-26e** (3 eq) Boc-Homoallyl Pro **3-26g** (3 eq)	$CHCl_3$ (0.2 M)	13%	$LiClO_4$ (1.4 eq)
7	Dimer **3-3** (1 eq) Boc-Homoallyl Phe **3-26e** (3 eq) Boc-Homoallyl Pro **3-26g** (3 eq)	$CHCl_3$ (0.3 M)	16%	$LiClO_4$ (1.6 eq)
8	Dimer **3-3** (1 eq) Fmoc-Homoallyl Phe **3-27a** (3 eq) Fmoc-Homoallyl Pro **3-27b** (3 eq) Fmoc-Homoallyl Gly **3-27c** (3 eq)	$CHCl_3$ (0.2 M)	18%	LiCl (7 eq)

3.3 Conclusions

Our main objective in this project was to examine the olefin cross-metathesis reactivity and selectivity of amino acid derivatives with cyclic scaffolds, and its potential for use in dynamic combinatorial chemistry. Having the olefin moiety further from the amino acid functional groups increased the yields of the homodimer products, but there were little differences in yields for the heterodimer products. Altering protecting groups on the amino acids from Boc to Fmoc resulted in little changes to the yields as well. The stereochemistry of the heterodimers was found to be predominantly trans, while that for the homodimers was found to be a mixture of cis/trans.

CHAPTER 4
DYNAMIC COMBINATORIAL LIBRARIES FROM PEPTIDOMIMETIC DIENES

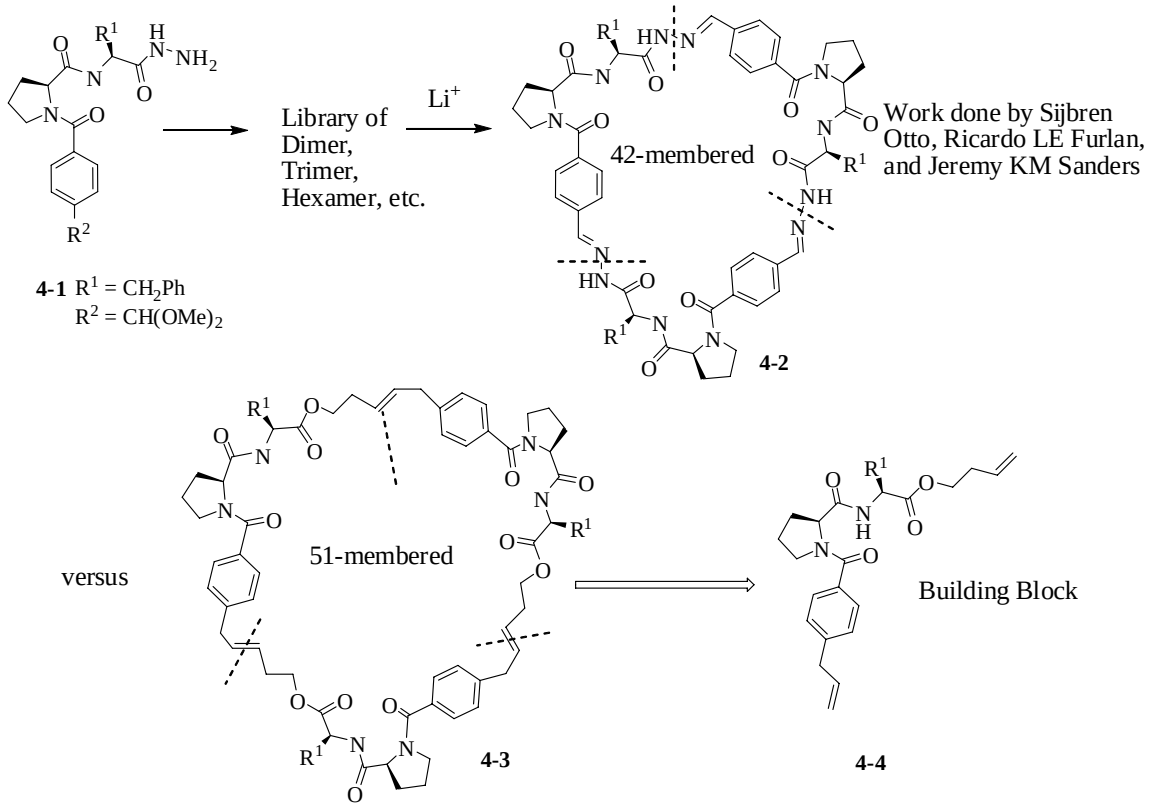
4.1 Introduction

In our continuing studies of cyclic peptidomimetics, we were interested in generating a dynamic combinatorial library[50,52,54,108] of macrocyclic compounds by olefin metathesis. Several groups have reported the use of reversible chemical reactions, such as disulfide exchange[55], metal-ligand coordination[56], exchange of oximes[57] and hydrazones[58], formal metathesis,[109] and olefin metathesis, in dynamic combinatorial chemistry.[59-61] However, few have examined olefin metathesis as a means to generate a library of cyclic peptidomimetics. The chemistry involves amino acid building blocks possessing two terminal olefin moieties. Cyclic molecules can be formed from these building blocks by ring-closing metathesis (RCM), or by cross-metathesis (CM) followed by RCM. A large number of library constituents, such as cyclic and linear compounds, as well as oligomers can be generated from this method. Upon addition of a template with specific binding properties to the library constitutes, the equilibrium can shift to amplify one or two major products in good yields.

Sanders' group used building blocks **4-1** consisting of L-proline, L-phenylalanine and an aromatic linker which can engage in non-covalent interactions, H-bonding, Lewis acid-base, pi-pi and cation-pi interactions.[52,58,110] Hydrozone formation was used to create these libraries of linear compounds, oligomers, and cyclic molecules. Sanders was able to show amplification of one of their library constituents, trimer **4-2**, by adding a lithium metal ion as a template (Scheme 4-1). We did not expect a small metal to

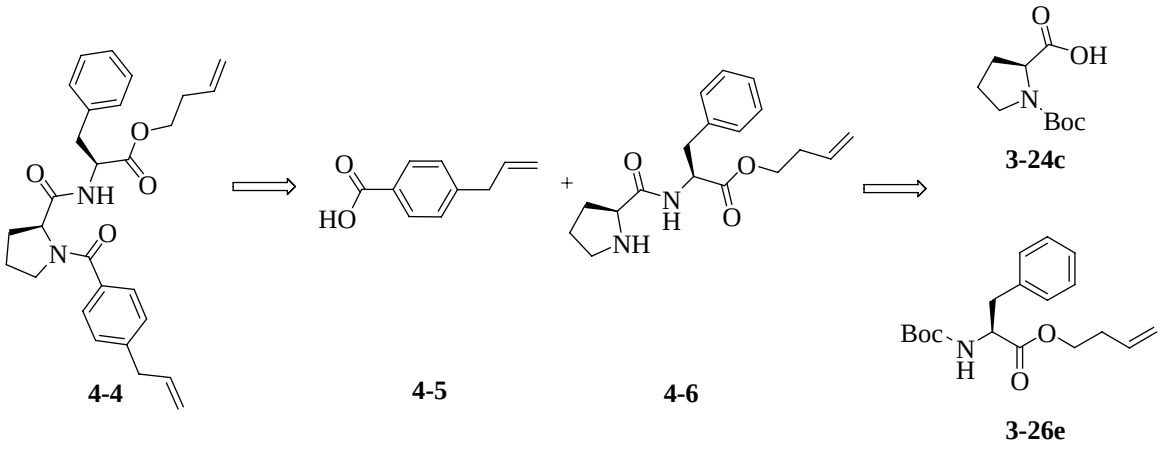
coordinate with the building blocks where the trimer was predominantly formed in a 1:1 complex with the metal.[52] Sanders employed other metals such as KI, RbI, and CsI as templates but did not observe the same shift toward the trimer.[58] However, under the same hydrozone based library, Sanders observed the amplification of the trimer using alkylammonium salts.[108,111]

We were curious to see if similar results could be obtained using a comparable building block but with olefin metathesis rather than hydrozone exchange. A trimer made up of dipeptide **4-4** would consist of a 58 membered ring (Scheme 4-1). A 48 membered macrocyclic could also be made by replacing the homoallyl moiety on building block **4-4** with an allyl ester. However, previous studies in our lab have shown that allyl groups are more sluggish toward metathesis compared to longer chain olefins.



Scheme 4-1. Comparison of building blocks **4-1** and **4-3** and their library constituents

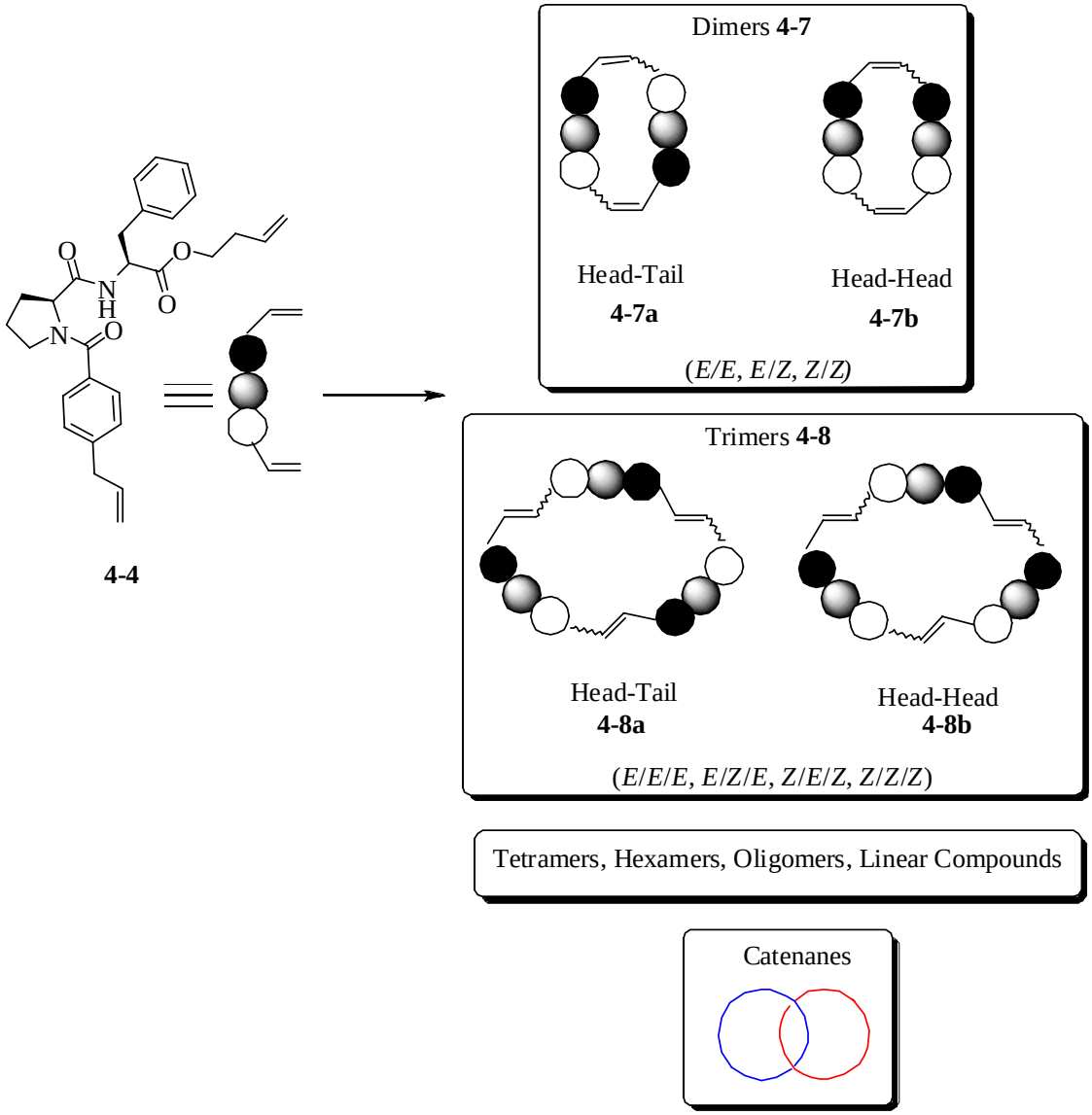
The proposed building blocks **4-4** would consist of the phenylalanine, proline, and aromatic compound and terminal alkenes to allow olefin CM and/or RCM. The retrosynthesis of dipeptide **4-4** is shown in Scheme 4-2. The molecule can easily be synthesized by the coupling of allyl benzoic acid **4-5** and peptide **4-6**, which is made from proline **3-24c** and phenylalanine **3-26e**.



Scheme 4-2. Retrosynthesis of dipeptide **4-4**

The CM and/or RCM reactions of dipeptide **4-4** can give a library of linear compounds, cyclic dimers **4-7**, trimers **4-8**, hexamers, and oligomers (Scheme 4-3). The regioselectivity and stereochemistry of the metathesis reactions can also increase the number and types of library constituents as well. For example, the coupling of two molecules can occur in a head to tail or a tail to tail fashion, to form dimers **4-7a** and **4-7b** respectively. In addition, CM and RCM reactions can give you *cis* or *trans* alkenes.

Catenanes, interlocked molecular rings, can also be generated by intramolecular RCM mediated syntheses under thermodynamic control.[112] Two molecules can become interwined and the twofold RCM can result in the formation of rings covalently linked (Figure 4-1). Several groups have also demonstrated the template or hydrogen bond directed synthesis of catenanes in good yields using olefin metathesis.[113-116]



Scheme 4-3. Library of dimers, tetramers, hexamers, oligomers, linear compounds and catenanes

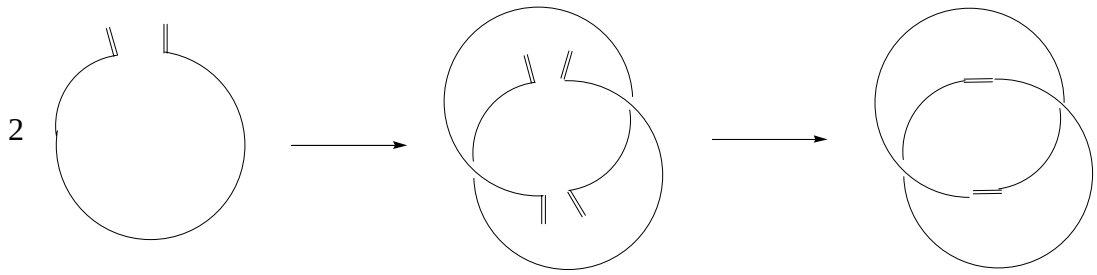
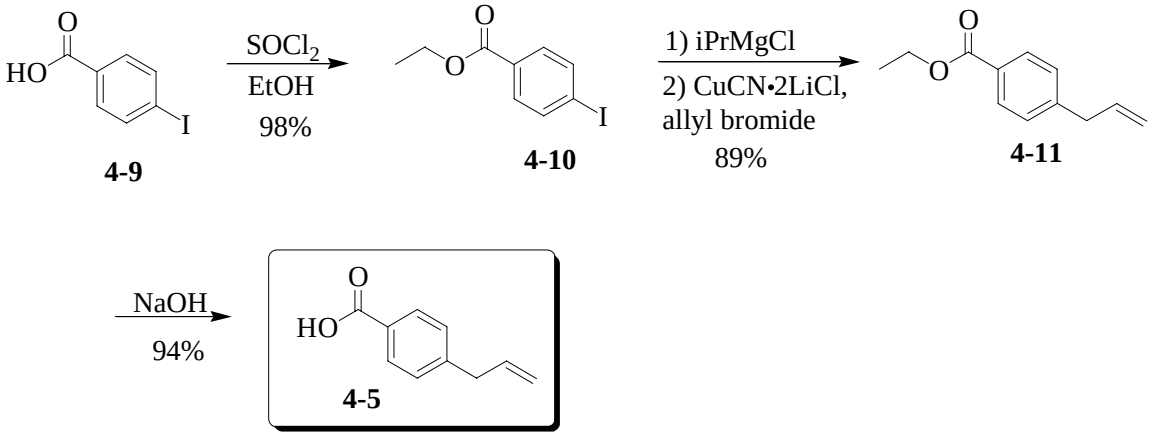


Figure 4-1. Formation of [2]catenane

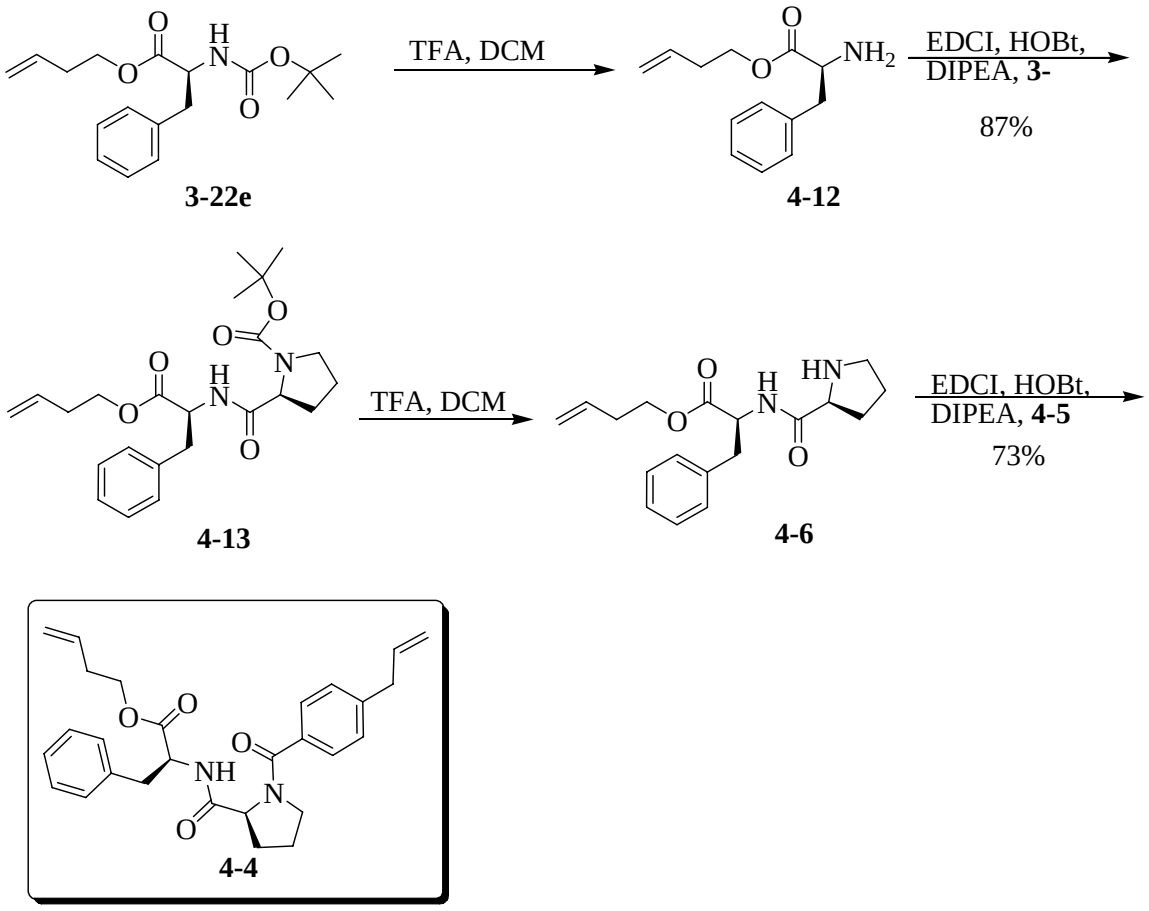
4.2 Results and Discussion

In order to study olefin CM and RCM in dynamic combinatorial library of cyclic peptidomimetics, we first needed to synthesize dipeptide **4-4**. The molecule can be synthesized by the coupling of 4-allyl benzoic acid (**4-5)** with peptide **4-6**. Allylbenzoic acid **4-5** was synthesized following literature procedures,[117,118] starting from commercially available 4-iodobenzoic acid (**4-9)** (Scheme 4-4). The synthesis involves esterification of acid **4-9**, followed by the conversion of the aryl iodide to the Grignard reagent. Treatment of the magnesium compound with allyl bromide and CuCN·2LiCl afforded benzoate **4-11**, which was then hydrolyzed to give 4-allyl benzoic acid (**4-5**) in excellent yield. [119]



Scheme 4-4. Synthesis of 4-allylbenzoic acid (**4-5**)

Dipeptide **4-4** was synthesized by a series of N-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDCI) amino acid coupling reactions (Scheme 4-5). Boc-homoallyl ester phenylalanine **3-26e** was first deprotected with TFA, and then coupled with Boc-L-proline **3-24c** to afford compound **4-13**. The Boc protecting group was removed with TFA once again, followed by another EDCI coupling reaction with allylbenzoic acid **4-5** to produce dipeptide **4-4** in good yield.



Scheme 4-5. Synthesis of dipeptide **4-4**

Upon the synthesis of dipeptide **4-4**, a series of CM experiments were conducted in various conditions (Table 4-1) to generate a library of peptidomimetics. We were interested in seeing the major components of the reaction when dipeptide **4-4** was allowed to react with catalyst **1-4**, with and without a template. A representative procedure for the CM reactions listed in Table 4-1 (experiment 11) is as follows: a solution of LiI (1 eq) and THF (2 mL) was added to a solution of dipeptide **4-4** and CH_2Cl_2 (20 mL). After stirring for 30 minutes, a solution of Grubbs' catalyst **1-4** and CH_2Cl_2 was added dropwise and the reaction refluxed for 13 h. Upon cooling, the reaction was quenched with EVE. The work-up included an aqueous extraction with tris(hydroxymethyl)phospine (THP) to remove the ruthenium catalyst.[105]

Table 4-1. Series of CM reactions

Experiment	Solvent (mM)	Catalyst **1-4** mol%	Template (equivalent)	Conditions
1	CH_2Cl_2 (5)	5	None	Monitored reaction for 4 days at r.t.
2	CH_2Cl_2 (36)	5	None	Monitored reaction for 4 days at r.t.
3	CH_2Cl_2 (5)	5	None	15 h at reflux
4	CH_2Cl_2 (5)	5	None	15 h at reflux
5	CH_2Cl_2 (5)	5	None	19 h at reflux
6	$CHCl_3$ (5)	5	None	19 h at reflux
7	THF (5)	5	None	Monitored reaction for 3 days at r.t.
8	THF (36)	5	None	Monitored reaction for 3days at r.t.
9	CH_2Cl_2 (5)	5	$LiClO_4$ (1.5)	Monitored reaction for 4 days at r.t.
10	CH_2Cl_2 (36)	5	$LiClO_4$ (1.5)	Monitored reaction for 4 days at r.t.
11	CH_2Cl_2 (5)	5	LiI (1 eq)	13 h at reflux
12	CH_2Cl_2 (5)	5	LiI (1 eq)	15 h at reflux
13	CH_2Cl_2 (5)	5	LiI (1 eq)	15 h at reflux
14	CH_2Cl_2 (5)	5	LiI (1 eq)	15 h at reflux
15	$CHCl_3$ (5)	5	LiI (1 eq)	13 h at reflux

The samples were analyzed on a Shimazu HPLC using a reverse phase column. The HPLC conditions consisted of an isocratic (65% acetonitrile, 35% water) flow rate of 0.5 mL/min and UV detection at 254 nm. HPLC spectra of selected experiments can be found in appendix a.

For experiments 1-2, the reaction mixture was stirred at room temperature in CH_2Cl_2 for 4 days (Table 4-1). There were only minor changes in the HPLC trace whether the concentration was 5 mM or 36 mM. Starting material was the major component after 4 days. We then examined THF as a solvent because it was later used to

dissolve the templates and we wanted to make sure THF wasn't a contributor to the equilibrium shift. Similarly to experiments 1-2, starting material was the major component when THF was employed both at concentrations of 5mM and 36mM (experiments 7 and 8, respectively).

The minor product formation was most likely due to the entropy of activation required to bring two terminal alkene moieties together for CM or ring closure .[28] Therefore, heat was employed and we conducted experiments 3-5, where the reaction mixture was refluxed in CH_2Cl_2 for 15 h and at 19 hr. There were little differences in the HPLC traces between the two reaction times. However, we observed new products compared to reactions maintained at room temperature. When $CHCl_3$ was used as the solvent, one major peak was detected.

We then examined the effects of $LiClO_4$ and LiI as template in various conditions. As shown in Figure 4-2, there was a decrease in peak F and an increase in peak J when $LiClO_4$ was employed and the reaction was maintained at room temperature. However, the starting material (peak D) was predominantly present in the reaction mixture. No major significant shift in the equilibrium was detected between experiments 2 and 10, when the reaction was maintained at a higher concentration (Figure 4-3). The minimum changes to the equilibrium when $LiClO_4$ was employed may be due to reactions conditions at room temperature, rather than lack of template effects. Therefore, we next examined the effects of lithium when the reaction was refluxed in CH_2Cl_2.

There was more of a dramatic change in the HPLC trace, when LiI was used as a template and the reaction was refluxed in CH_2Cl_2 (5mM) (Figure 4-4). There was a large decrease of peak D and an increase in some of the larger molecules (peaks G, H, and I).

LiI was chosen as the template rather than $LiClO_4$ for these set of experiments to better replicate Sanders' work.

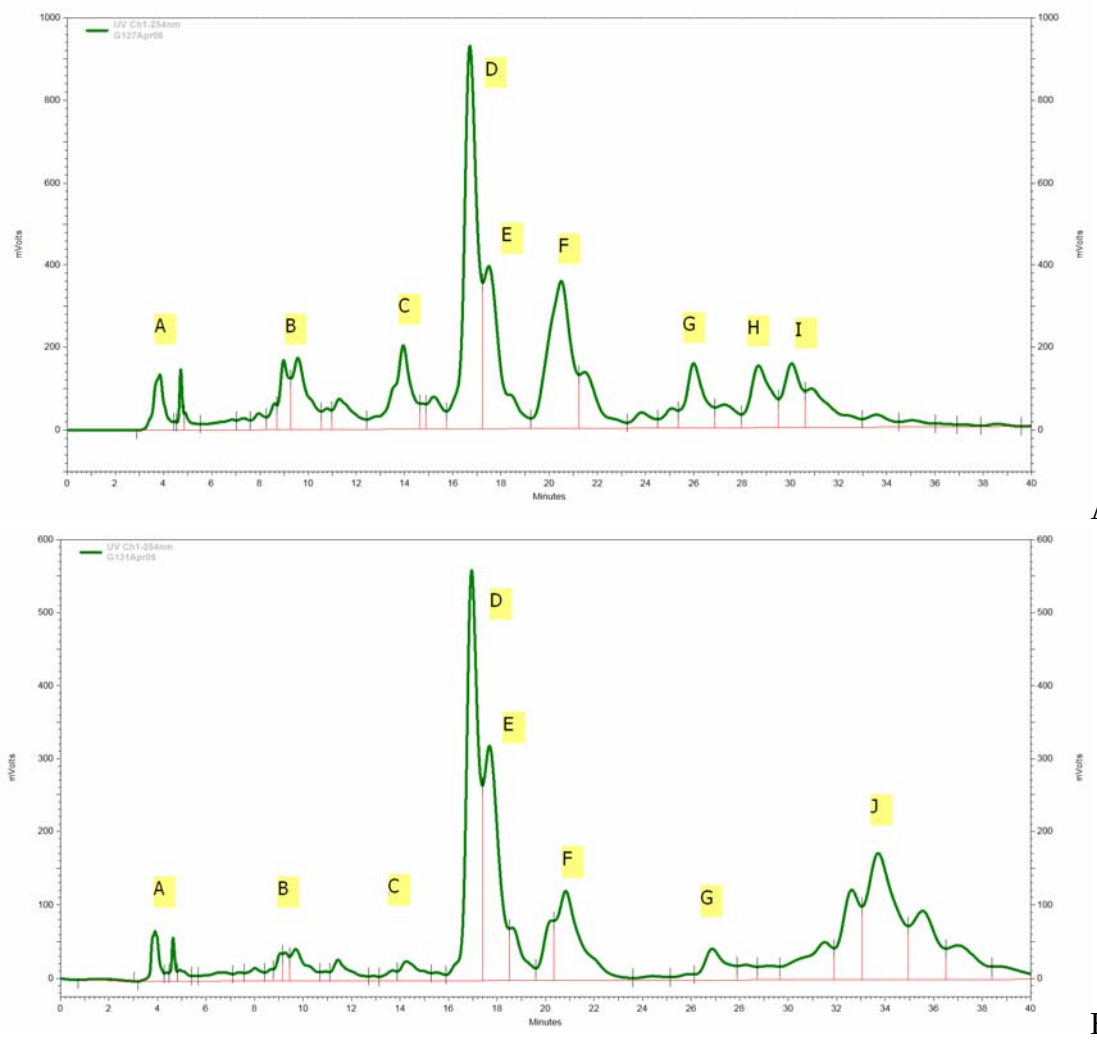


Figure 4-2. A) HPLC spectrum of Experiment 1 reaction mixture (CH_2Cl_2, 5mM, room temperature, 4 days, no template) and B) HPLC spectrum of Experiment 14 reaction mixture (CH_2Cl_2, 5mM, room temperature 4 days, $LiClO_4$)

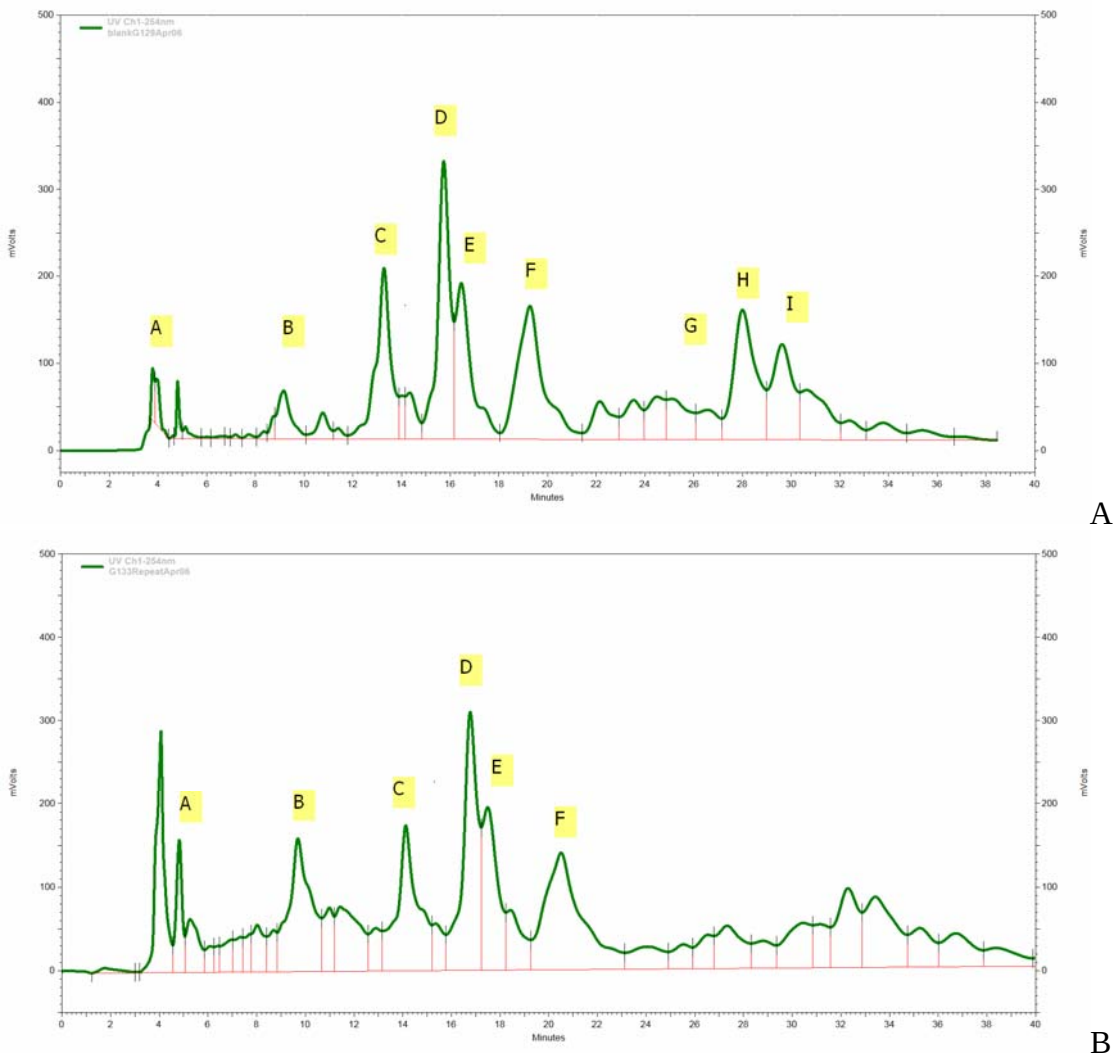


Figure 4-3. HPLC spectrum of Experiment 2 reaction mixture (CH_2Cl_2, 36mM, room temperature, 4 days, no template) and B) HPLC spectrum of Experiment 10 reaction mixture (CH_2Cl_2, 36mM, room temperature 4 days, $LiClO_4$)

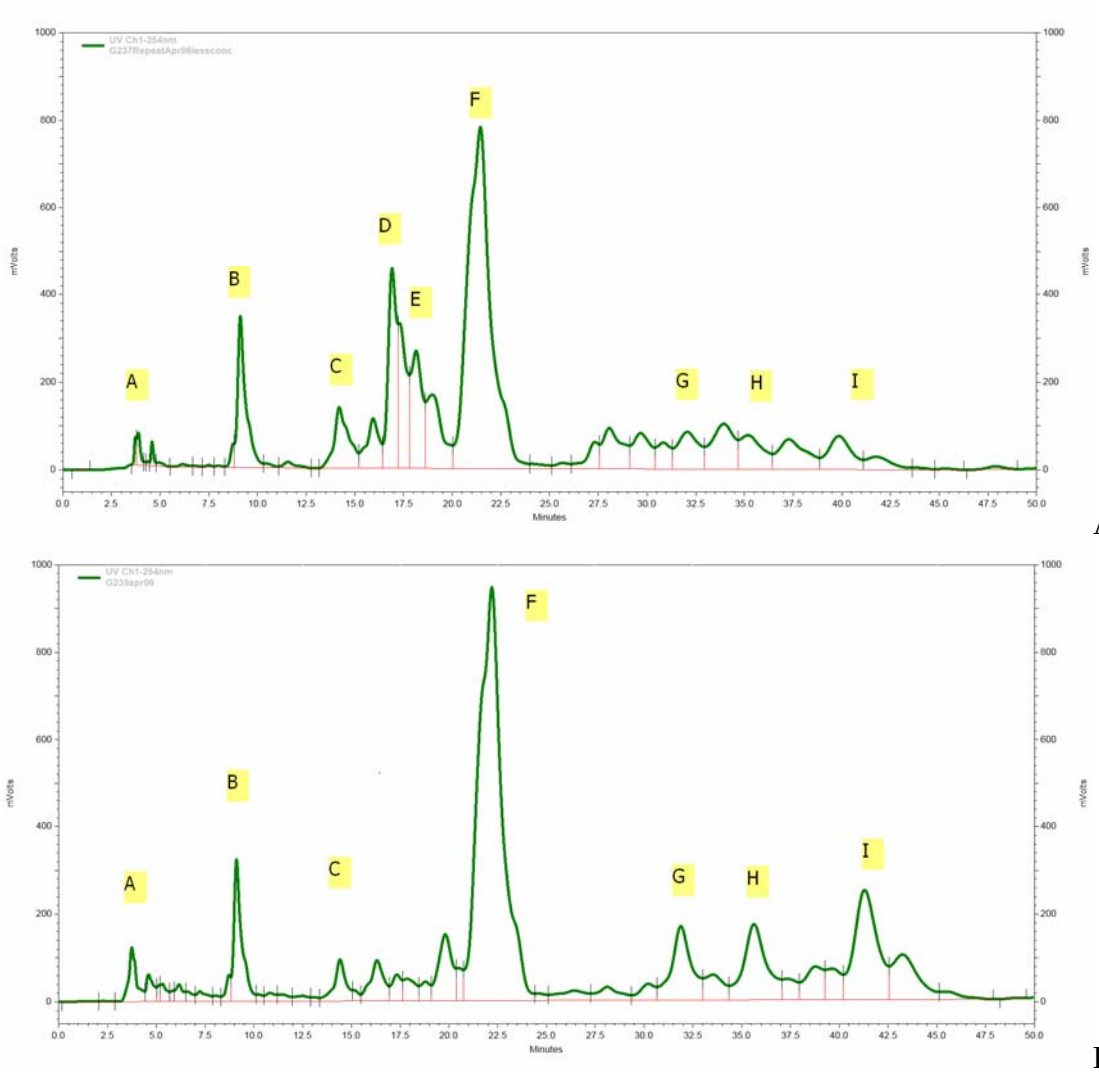


Figure 4-4. A) HPLC spectrum of Experiment 4 reaction mixture (CH_2Cl_2, 5mM, reflux 15 hours, no template) and B) HPLC spectrum of Experiment 13 reaction mixture (CH_2Cl_2, 5mM, reflux 15 hours, LiI)

A series of reactions were also monitored by HPLC where the dipeptide was allowed to react with catalyst **1-4** for a period of several hours to 24 hours at room temperature to allow equilibration. Afterwards, different lithium templates (LiI or $LiClO_4$) were added and the reaction maintained for another 24 h. We continued to monitor the reaction after refluxing for another 24 h. For some experiments, additional 5 mol% of the catalyst was introduced every 24 h to ensure the catalyst was not degraded.

However, we didn't observe any significant changes in the HPLC data when the lithium template was added before the catalyst to allow pre-coordination of the amino acids with the metal.

Even though we didn't see a significant shift in the equilibrium before and after the addition of the template as was seen in Sanders' work, we felt it was of interest to identify the major peaks. Attempts were made to isolate the major products by column chromatography. We were able to recover the starting material, dipeptide **4-4**. However, obtaining pure CM products was more difficult than anticipated. We did isolate a compound that appeared to be a dimer made up of two molecules of dipeptide **4-4** based on HRMS and 2D NMR [(acetone d_6), COSY (Correlated Spectroscopy), HMBC (Heteronuclear Multiple Bond Correlation), HMQC (Heteronuclear Multiple Quantum Correlation), NOESY (Nuclear Overhauser and Exchange Spectroscopy)]. The ^{1}H and ^{13}C spectra were obtained on an Inova spectrometer operating at 500 MHz for proton and 100 MHz for carbon. Two dimensional NMR experiments were conducted on the same spectrometer. Samples were dissolved in deuterated acetone. The HPLC trace of the isolated compound is shown in Figure 4-5.

HRMS data of the isolated compound revealed three possible structures as shown in Figure 4-6. The dimer of dipeptide **4-4** can be formed by the CM of two molecules in a head to head or head to tail fashion, followed by a RCM to give **4-7a** and **4-7b**, respectively. In addition, the HRMS data also corresponded with the structure of [2]catenane **4-14**, in which two rings of dipeptide **4-4** are interlocked within each other (Figure 4-6).

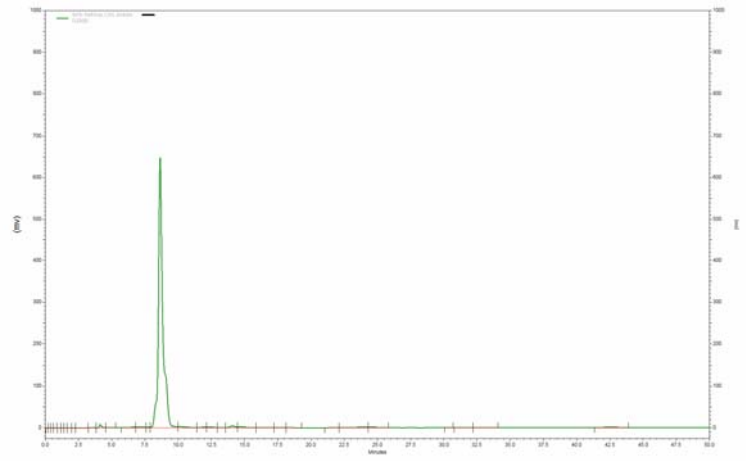


Figure 4-5. HPLC spectrum of isolated compound from Experiment 3

Theoretical m/z: $C_{52}H_{57}N_4O_8$ [M+H]+ = 865.4171
Found: $C_{52}H_{57}N_4O_8$ [M+H]+ =865.4174

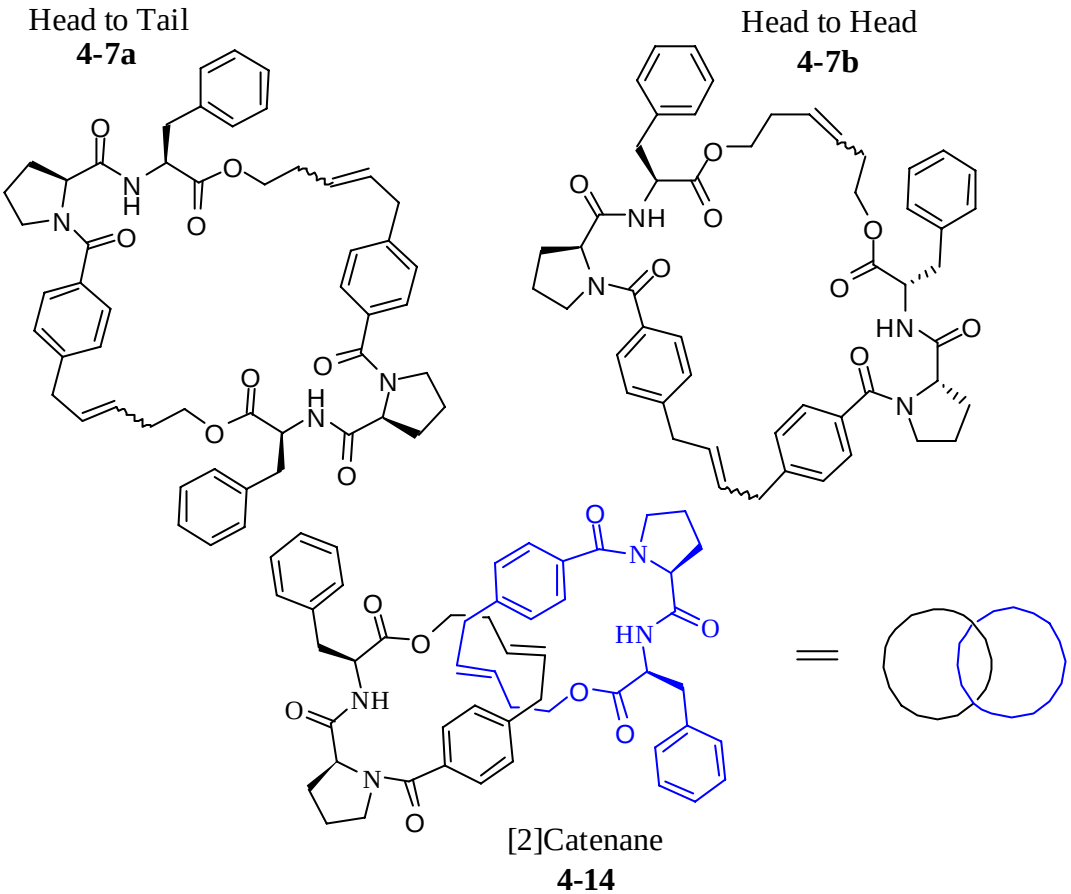
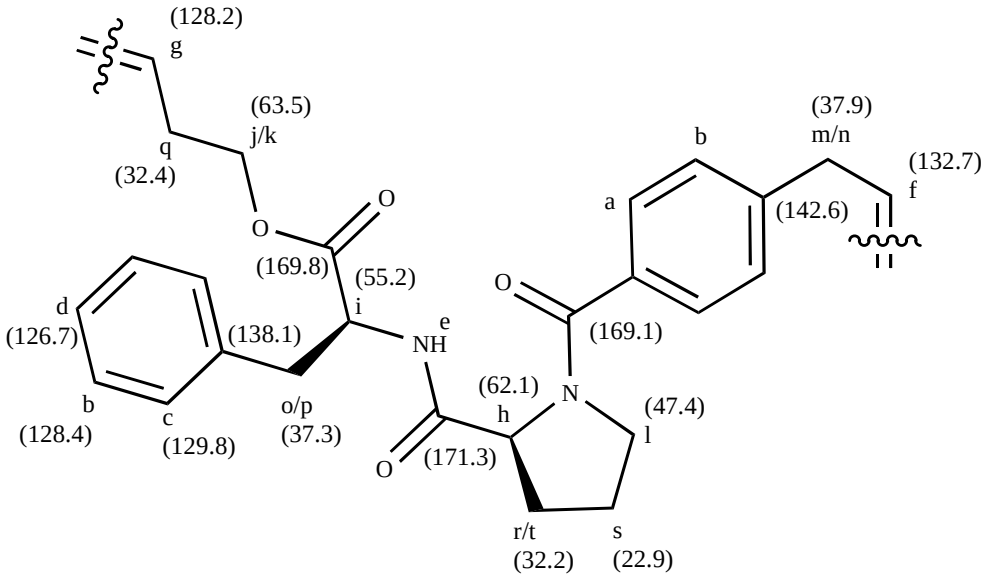


Figure 4-6. Structures of dimers **4-7a** and **4-7b**, and catenane **4-14**

The NMR chemical shifts and coupling values of the isolated compound are shown in Figure 4-7 and Table 4-2. NMR data proved that two dipeptide building blocks were attached in a head to tail fashion because the alkene protons had two different chemical shifts (5.04 and 5.84 ppm), which ruled out structure **4-7b**. If the building blocks were linked in a head to head fashion, then the chemical shifts would be identical.



Numbers in parenthesis refer to ^{13}C chemical shifts

Figure 4-7. ^{13}C Chemical shifts and the corresponding protons of isolated compound

To determine if the isolated product was structure **4-7a** or catenane **4-14**, we examined the NOESY spectrum. Molecular models of catenane **4-14** were generated using the HyperChem program. The most likely conformation of catenane **4-14** is shown in Figure 4-8, where the phenyl group on one ring is perpendicular to the alkene of the second ring. Because of the shielding effects of the phenyl ring on the olefinic protons, we would expect the chemical shifts to be approximately 2-3 ppm further upfield.[104] However, we did not observe the shielding effects on the olefinic protons. Therefore,

catenane **4-14** can be ruled out, leaving the most likely structure to be dimer **4-7a** (Figure 4-9). In addition, the large *J* value (15 Hz) indicated the presence of a *trans* alkene.

Table 4-2. Proton chemical shifts and J values

Proton	Chemical Shift (ppm)	Spin Coupling (*J* value in Hz)[1]
a	7.39	m
b	7.26	m
c	7.22	m
d	7.18	m
e	6.62	d (7.2)
f	5.84	d,t,t (15.3, 6.0, 1.1)
g	5.04	dddt (14.3, 7.9, 6.2, 1.6)
h	4.55	dd (8.3, 3.5)
i	4.28	q (7.3)
j	4.27	m
k	3.82	m
l	3.62	m
m	3.38	dd (15.1, 6.6)
n	3.22	dd (14.5, 6.2)
o	3.02	dd (14.1, 7.6)
p	2.66	dd (13.7, 7.7)
q	2.25	m
r	2.18	m
s	1.84	m
t	1.76	m

[1]doublet (d), multiplet (m), triplet (t), q (quartet)

In addition to dimer **4-7a**, we also attempted to isolate some other major CM products by column chromatography. We obtain a compound that corresponded to peak X of the HPLC trace shown in Figure 4-10, however purification was not successful. HRMS of the compound showed the presence of a dimer made up of two dipeptide **4-4** molecules. However, ^{1}H NMR of the isolated compound appeared oligomeric and 2D NMR confirmed the presence of terminal alkenes. Therefore, we could only conclude that there was a mixture of CM products corresponding to peak X.

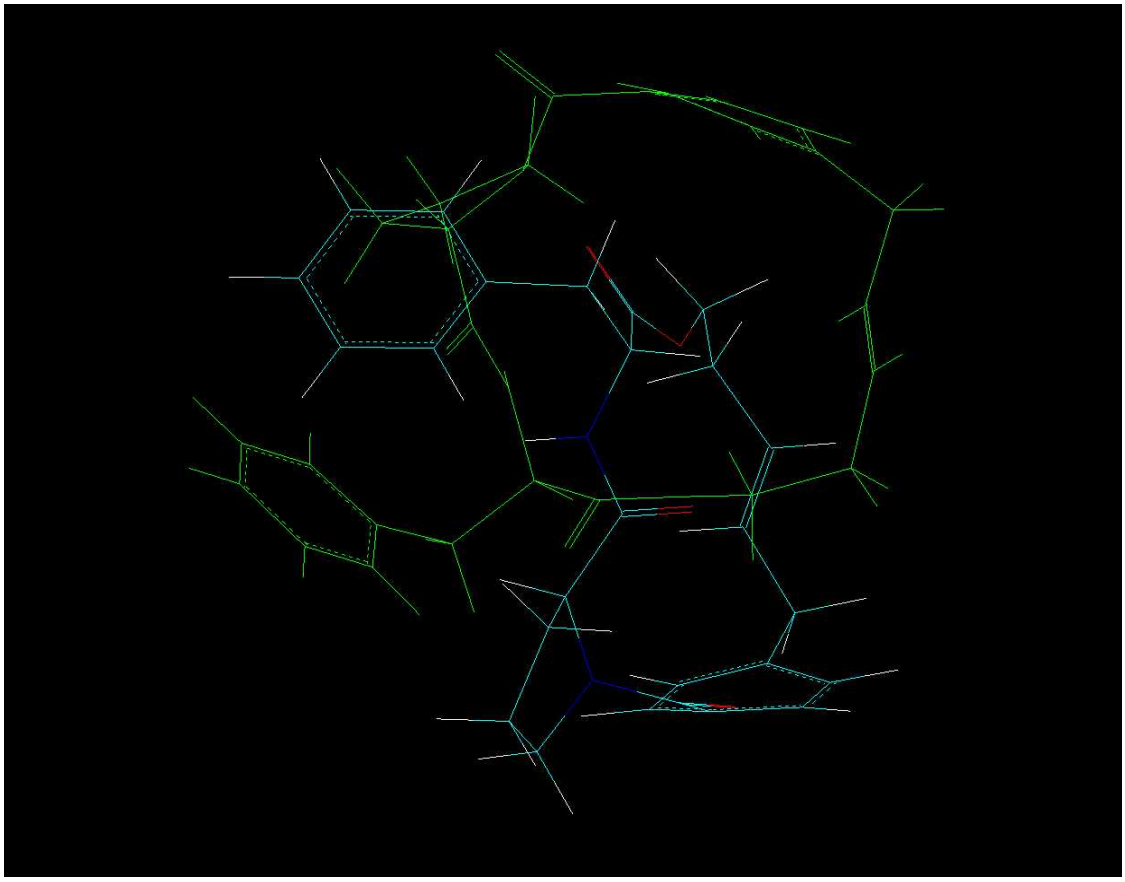


Figure 4-8. Model of catenane **4-14**

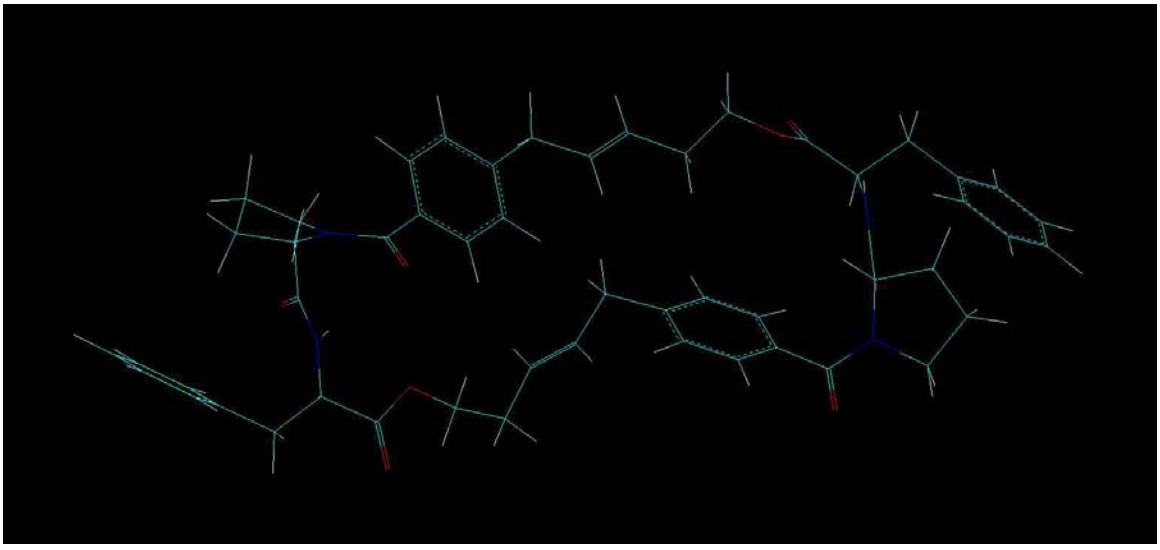


Figure 4-9. Model of dimer **4-7a**

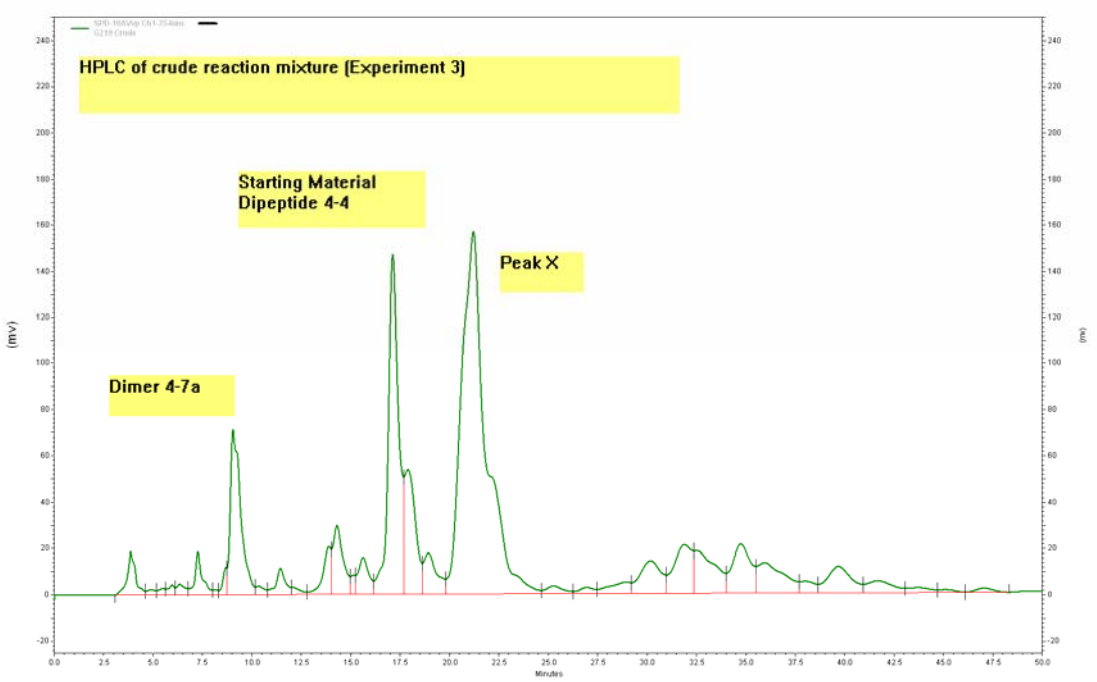


Figure 4-10. HPLC spectrum of Experiment 4 reaction mixture (CH_2Cl_2, 5mM, reflux 15 hours, no template

4.3 Conclusions

A series of CM reactions were conducted to generate a library of peptidomimetics. The building block consisted of a dipepetide molecule consisting of a phenylalanine, proline, aromatic linker, and two terminal olefin moieties. Lithium templates were introduced into the system to determine the influence on the equilibrium. There was no dramatic shift in the equilibrium where one major product was formed as demonstrated by Sanders' group. However, the largest change occurred in our system when the reaction mixture was refluxed in CH_2Cl_2 (5mM) for 15 hours and LiI was used as the template. We have isolated and characterized dimer **4-7a**, which was formed by the CM and RCM of dipeptide **4-4** in a head to tail arrangement with the *trans* alkene favored. The research group is continuing the project to isolate and purify other peptide molecules.

CHAPTER 5
MODEL STUDY - MOLECULAR IMPRINTING OF NERVE GASES

5.1 Introduction

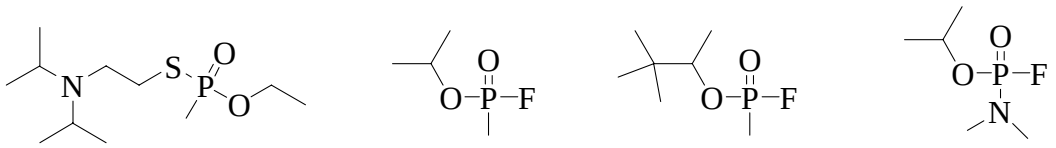
Molecular imprinted polymers (MIPs) are useful as mimics of biological receptors, enzymes, and antibodies.[69,70] Their application in chemical sensors is imperative due to national concerns over chemical warfare. This current threat involves the use of chemical agents that can incapacitate or kill those who are exposed by affecting the functions of the body, such as the nervous system, lungs, blood, or skin. [120]

Chemical weapons were first used during World War I by the Germans. During an attack against the Allied troops, the release of chlorine gas resulted in an estimated 5,000 dead and 10,000 disabled.[121] Chemical weapons are not just a concern for the military, but also civilians. In 1981, the Iraqis attacked a Kurdish town with mustard gas, a blister agent that can cause nausea, vomiting, pain, and death[121]. Due to recent terrorist attacks on U.S. soil, the ability to detect these deadly agents quickly and accurately has been a priority in national defense.

Our group is interested in using molecular imprinted technology as a sensor for chemical agents such as the nerve gases (Figure 5-1). These organophosphates interfere with the nervous system and can cause injury or death. Of the four chemical agents shown in Figure 5-1, VX (**5-1**) is the most deadly.[120]

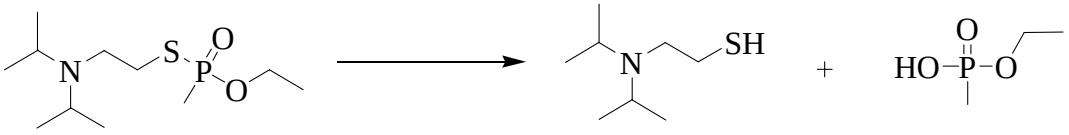
Due to the toxicity of VX, we have chosen 2-(diisopropylamino)ethanethiol (**5-5**) as the template. The thiol is a degradation product of VX and less hazardous to handle[122].

Thiol **5-5** can be synthesized from thiourea and 2-(diisopropylamino)ethyl chloride following literature procedures.[123]



VX **5-1** Sarin **5-2** Soman **5-3** Tabun **5-4**

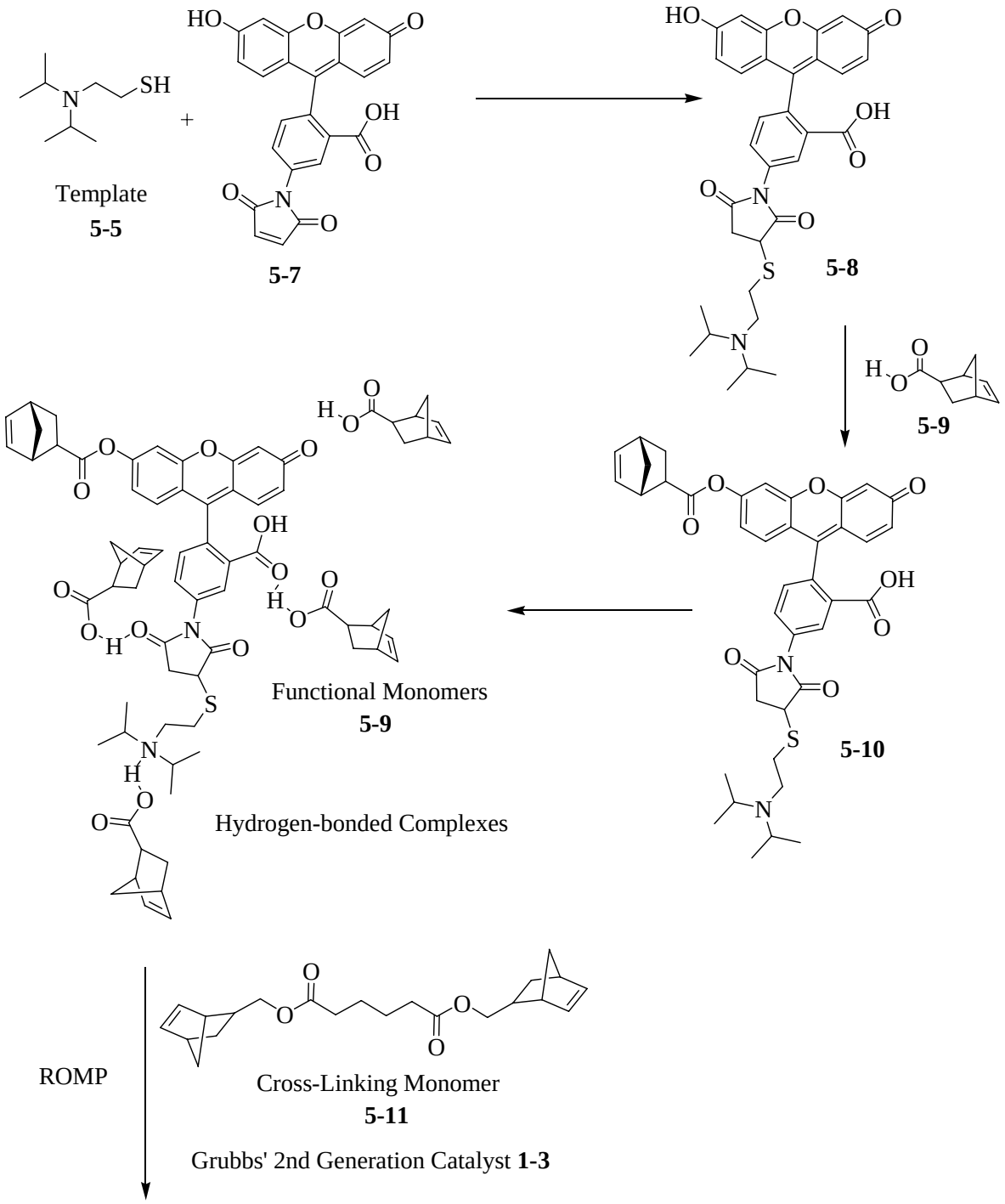
Figure 5-1. Nerve agents



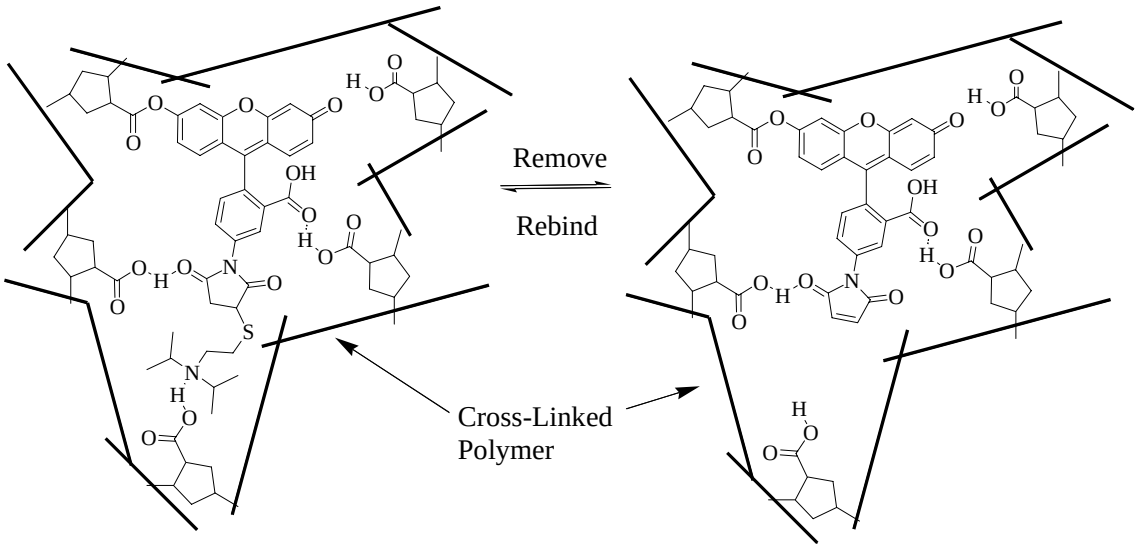
5-1 **5-5** **5-6**

Scheme 5-1: Degradation Products of VX

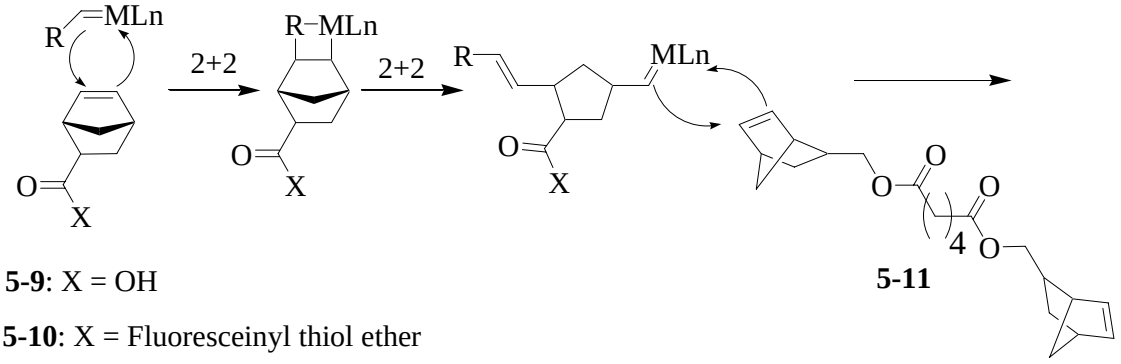
The development of MIP involves the Michael addition of thiol template **5-5** to the fluorescein maleimide **5-7**, which will serve as the dye (Scheme 5-2). Compound **5-8** will be coupled to 5-Norbornene-2-carboxylic acid (**5-9**, mixture of endo and exo), which is required for polymerization. Carboxylic acid **5-9** will all serve as the functional monomer and will be allowed to form hydrogen-bonded complexes with **5-10**. Ring Opening Metathesis Polymerization (ROMP) with the complex and cross-linking monomer (**5-11**) using Grubbs' second generation catalyst **1 -3** will result in the rigid network (Schemes 5-2 and 5-3). The mechanism of the ROMP with the monomers and dye is shown in Scheme 5-4. Removal of the template and the regeneration of the maleimide will leave the desired imprint. In other words, a molecular memory will be introduced in the MIP. The recognition sites will possess the correct shape and orientation of the functional groups to make it selective for thiol template **5-5**.



Scheme 5-2. Formation of hydrogen-bonded complexes

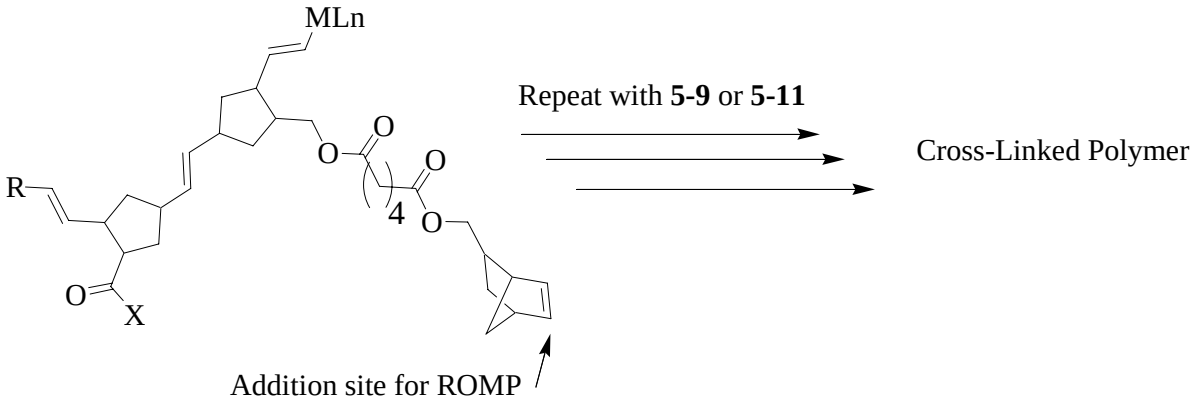


Scheme 5-3. Formation of the MIP



5-9: X = OH

5-10: X = Fluoresceinyl thiol ether

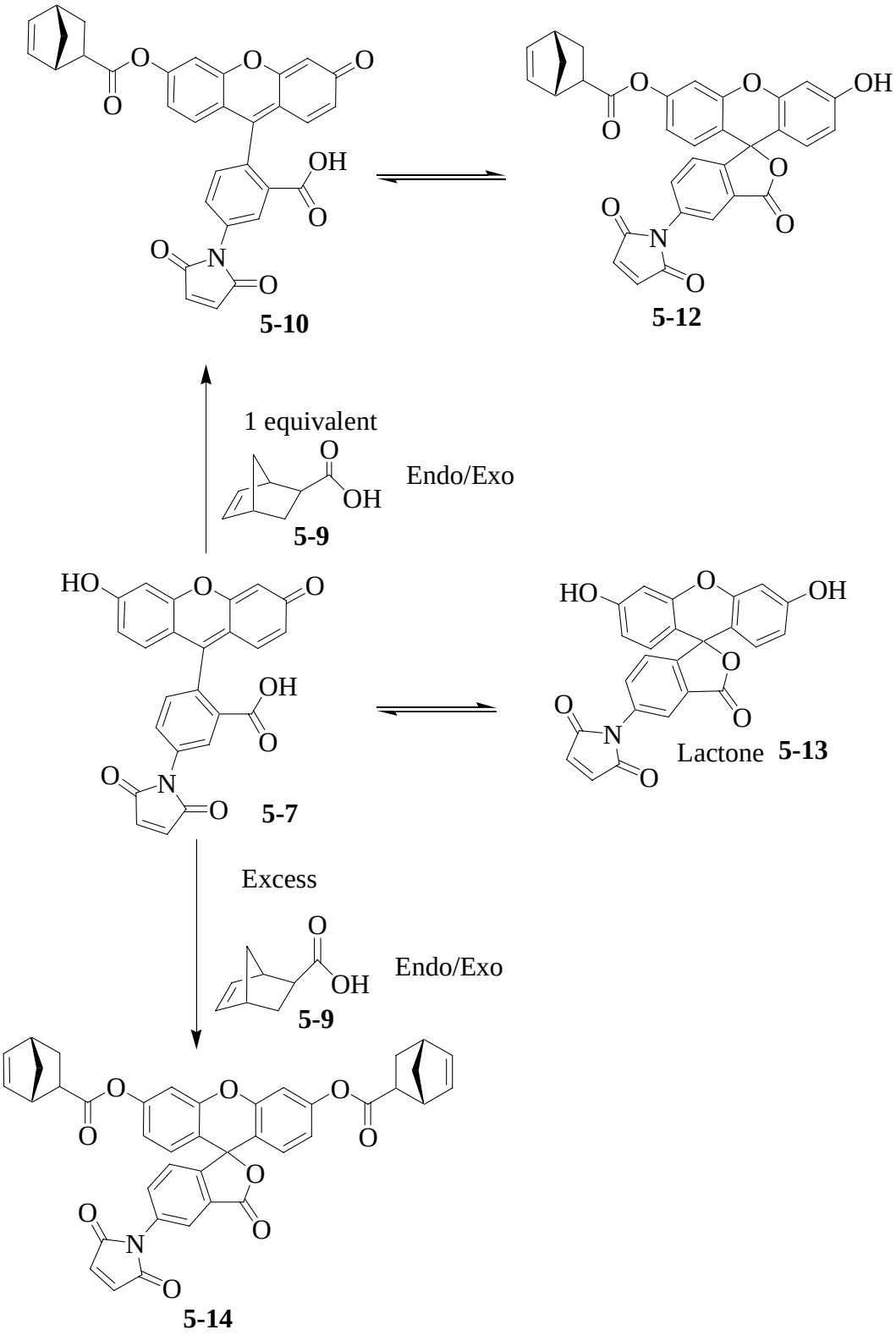


Scheme 5-4. Mechanism of the ROMP polymerization.

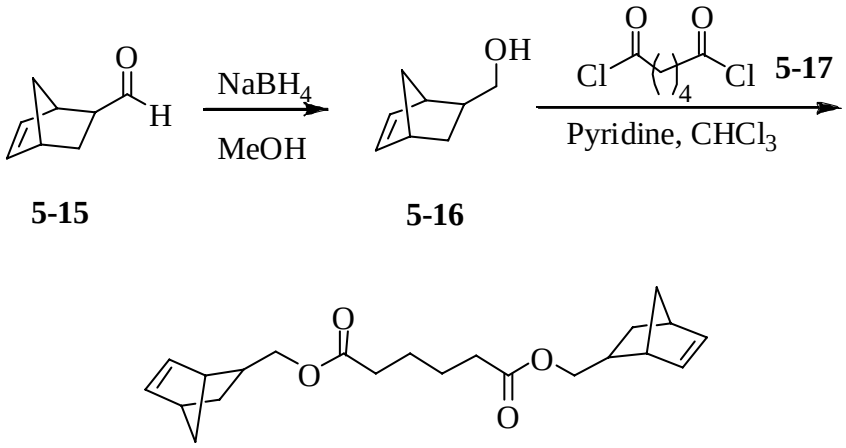
Florescein maleimide **5-7** was chosen as the dye because it has been extensively used as a thiol reactive dye.[124-126] It possess many useful photophysical properties and is very reactive and selective toward thiol groups.[124,127] It absorbs light of mean wavelength 427 nm and emits visible green light at 515 nm.[124] One research group demonstrated the increase in fluorescence emission when glutathionine, a tripeptide containing a thiol group, was allowed to react with fluorescein maleimide.[128] We plan on examining the reaction of our thiol substrates with the dye and determining the effects on the fluorescence emission. Fluorescein maleimide was also selected as the dye because the norbornene moiety, which is required for ROMP, can be coupled to either one or two of the alcohol groups in one step without modification to the dye (Scheme 5-5). With only one norbornene molecule attached, the fluorescein molecule can exist in two tautomeric forms.

Cross Linking monomers **5-11** have been made previously in our group, starting from commercially available 5-norbornene-2-carboxaldehyde (**5-15**).[73] The aldehyde can be reduced to alcohol **5-16**, which can then react with adipoyl chloride (**5-17**) to form diester **5-11** (Scheme 5-6).

The ratios of the template/monomer/cross-linking agent will be tested to determine the best polymerization conditions. Binding properties of the MIP will be evaluated using gas chromatography and mass spectrometry methods to ensure that it is specific to only thiol **5-5.**



Scheme 5-5. Coupling of norbornene moiety to fluorescein maleimide

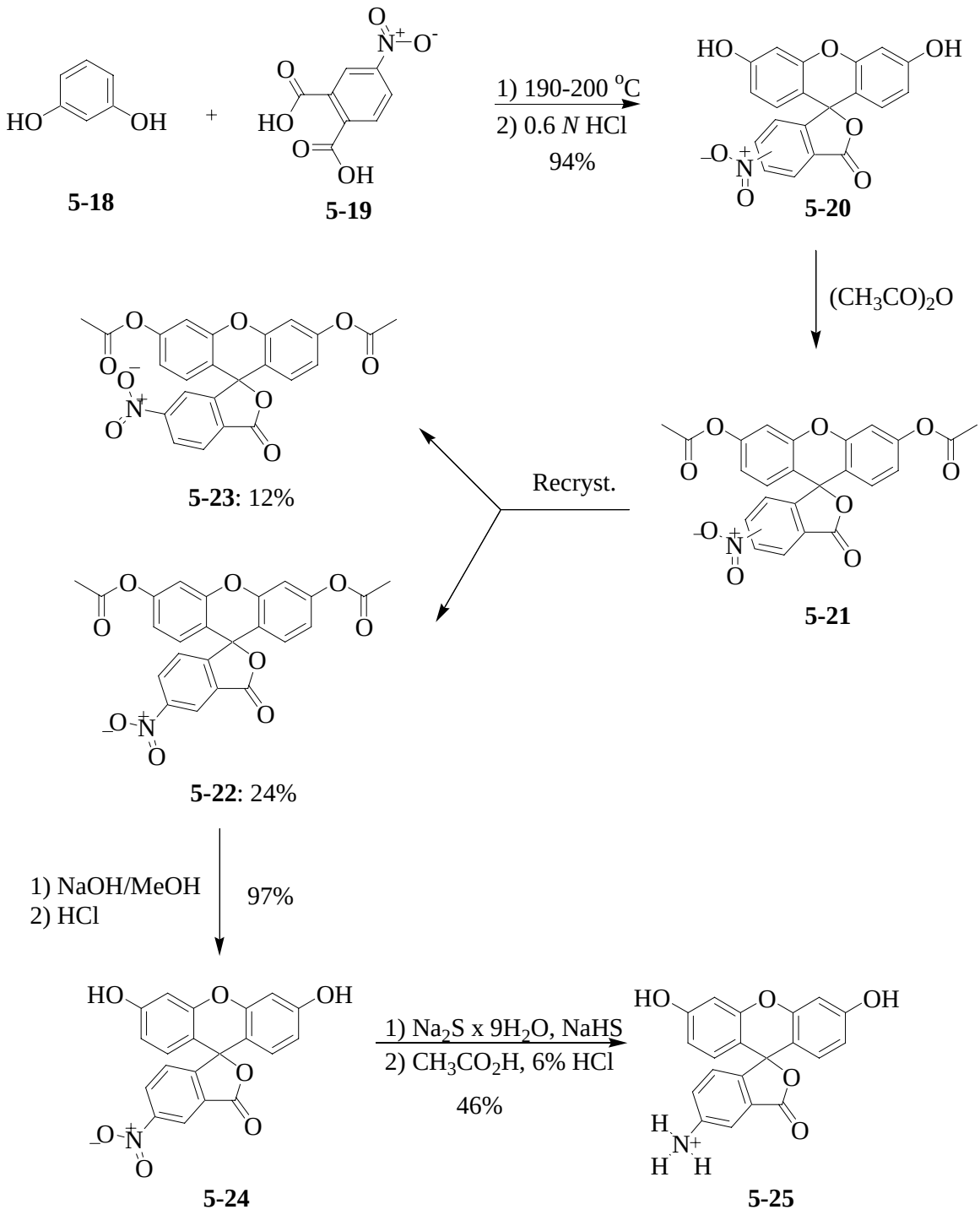


Scheme 5-6. Synthesis of cross-linking monomer **5-11**

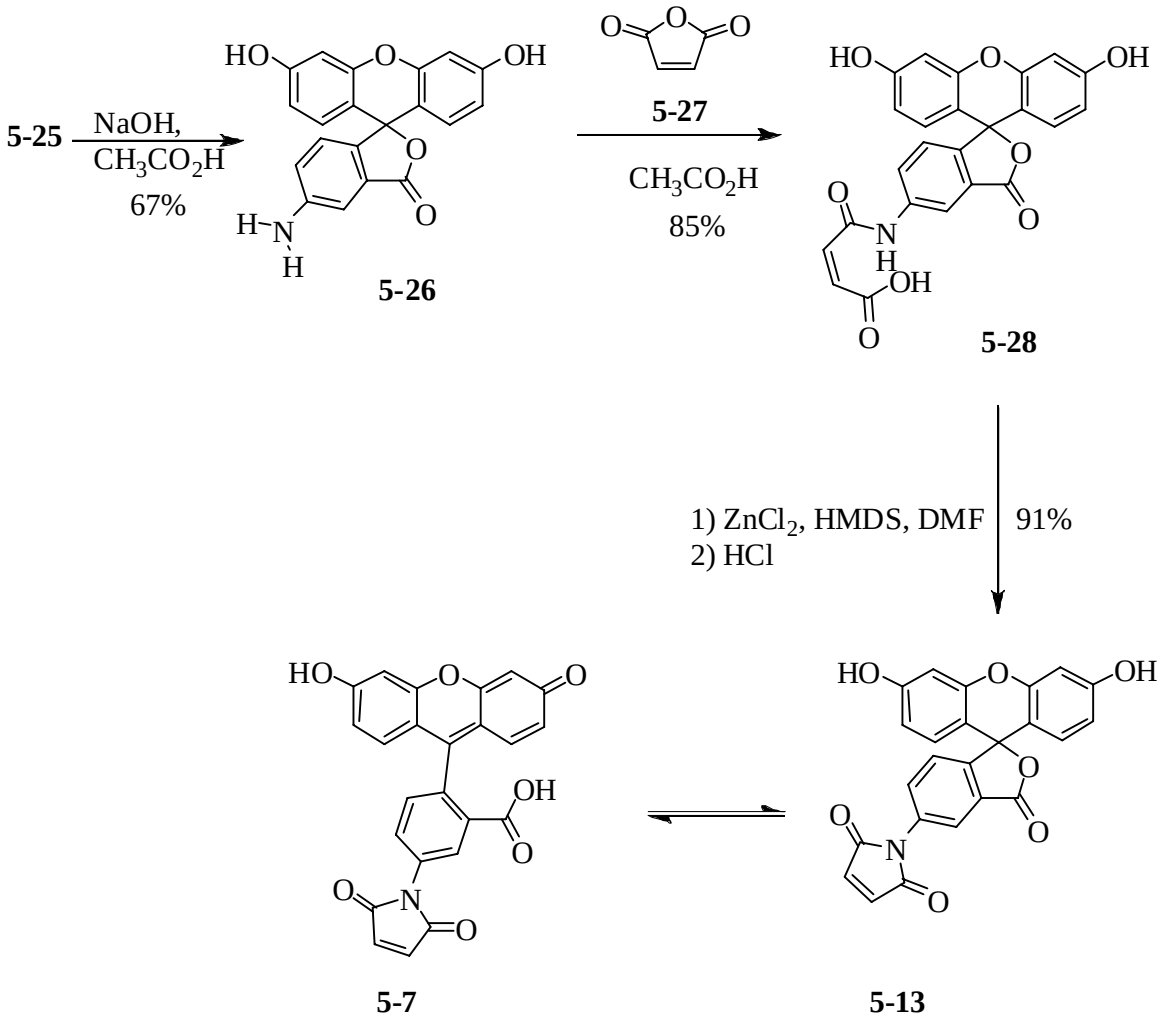
5.2 Results and Discussion

5.2.1 Synthesis of N-(5-Fluoresceinyl)maleimide **5-7**

N-(5-Fluoresceinyl) maleimide (**5-7**) is commercially available, but also very expensive. Therefore, we decided to synthesize the maleimide based on a combination of literature procedures (Schemes 5-7 and 5-8).[127,129-132] The synthesis involves 5 major steps: 1) Condensation of commercially available resorcinol (**5-18**) and 4-nitrophthalic acid (**5-19**) to form 5-6-nitrofluorescein **5-20**; 2) Acetylation and crystallization to isolate isomers **5-22** and **5-23**; 3) Saponification of isomer **5-22** to give desired 5-nitrofluorescein (**5-24)**; 4) Reduction of **5-24** with sodium sulfide nonahydrate ($Na_2S.9H_2O$) and sodium hydrosulfide (NaHS) to produce 5-aminofluorescein (**5-26)**; 5) Reaction of aminofluorescein **5-26** with maleic anhydride **5-27** in acetic acid to give maleic acid **5-28** and cyclization to yield fluorescein maleimide **5-7**.



Scheme 5-7. Synthesis of fluorescein amino hydrochloride **5-25**



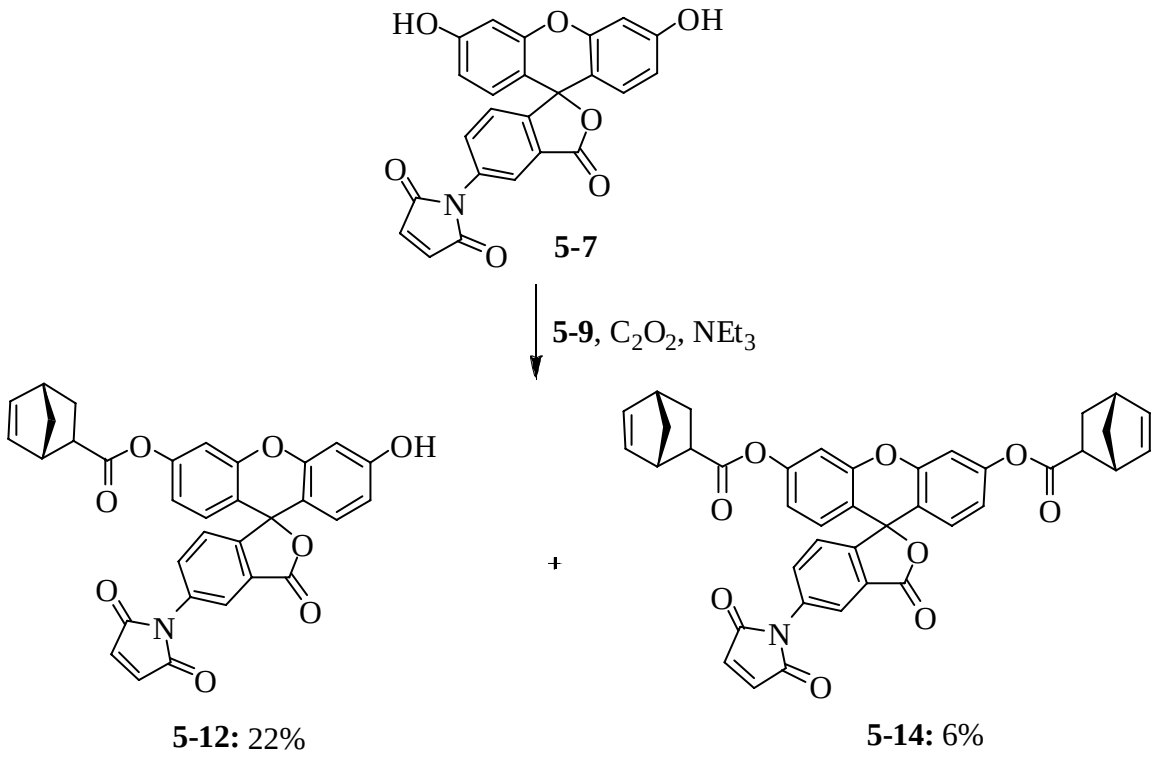
Scheme 5-8. Synthesis of N-(5-fluoresceinyl)maleimide (**5-7**)

Crude nitrofluorescein **5-20** was obtained in good yields by simply heating resorcinol (**5-18**) and 4-nitrophthalic acid (**5-19**) at very high temperatures. However, to isolate pure diacetate isomers **5-22** and **5-23**, several recrystallizations were required which resulted in low yields. Diacetate **5-22** was converted to 5-nitrofluorescein **5-24** through saponification methods with NaOH/MeOH. Based on work by McKinney[130] and Steinbach[132], $Na_2S.9H_2O$ and NaHS were used to reduce **5-24** to aminofluorescein **5-26,** rather than hydrogen and Raney nickel. Our attempts to isolate the aminofluorescein **5-26** over several recrystallizations resulted in lower than expected yields.

At this point, it was determined to be more time and cost efficient to purchase aminofluorescein **5-26** from Aldrich, and follow Reddy's procedures[127] to obtain maleimide **5-7**. If large quantities of aminofluorescein **5-26** are required than it would be more cost beneficial to start from compounds **5-18** and **5-19**. Maleimide **5-7** was easily synthesized in good yields by reacting aminofluorescein **5-26** in glacial acetic acid and maleic anhydride **5-27**, followed by cyclization by use of the Lewis acid Zn_2Cl and hexamethyldisilazane (HMDS).

5.2.2 Synthesis of Compounds **5-12** and **5-14**

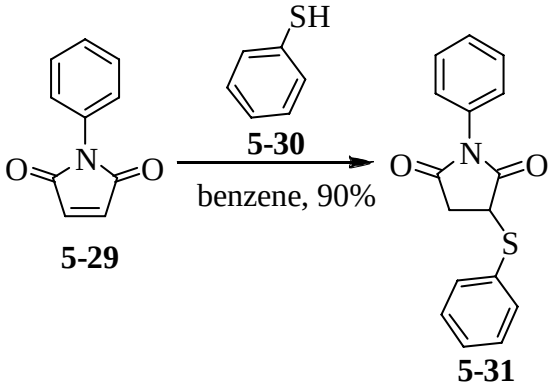
To ensure we could couple the norbornene moiety to the fluoresceinyl dye, we investigated the synthesis of compound **5-12**. Carboxylic acid **5-9** was treated with fluorescein maleimide **5-7** in the presence of dicyclohexylcarbodiimide (DCC) and dimethylamino pyridine (DMAP). However, it was difficult to remove the byproduct, dicyclohexylurea (DCC) even after filtration and column chromatography. Similar results were obtained using diisopropylcarbodiimide (DIC). Therefore, we decided to use oxalyl chloride (Scheme 5-9). Carboxylic acid **5-9** reacted with oxalyl chloride to form the acid chloride which was then dissolved in CH_2Cl_2. The acid chloride solution was added dropwise to a solution of maleimide **5-7**, CH_2Cl_2, and NEt_3 at 0 °C. Dilute conditions (0.03 M) were used to prevent over acylation. The mono-acylated **5-12** was isolated in 22% yield and the di-acylated **5-14** in 6% yield.



Scheme 5-9. Synthesis of compounds **5-12** and **5-14**

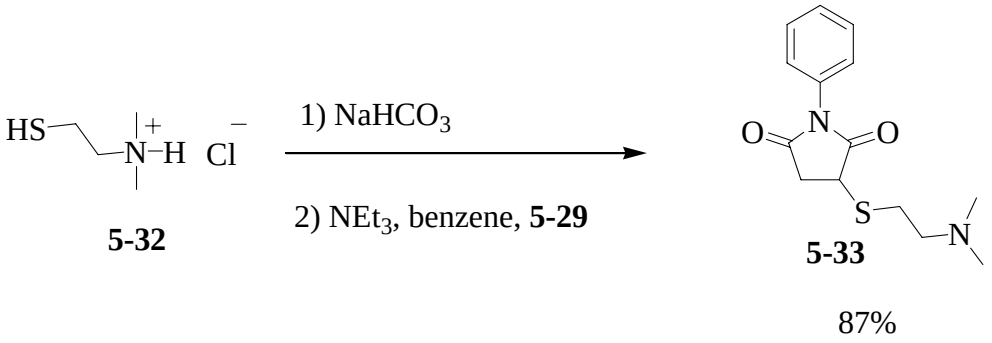
5.2.3 Model Study

We selected phenyl maleimide **5-29** as a model to test the reactivity of the Michael addition of benzenethiol (**5-30)** and the elimination of the thiol derivative to regenerate maleimide **5-7**. Following literature procedures,[133] phenylthiol succinimide **5-31** was synthesized in high yields (Scheme 5-10).



Scheme 5-10. Synthesis of succinimide **5-31**

We then tested the reactivity of phenyl maleimide **5-29** with dimethylaminoethanethiol salt **5-32**, which is similar to our target molecule **5-5**. The thiol salt **5-32** was deprotonated with Na_2CO_3, and the addition to the double bond of the maleimide gave thioether **5-33** in 87% yield (Scheme 5-11).



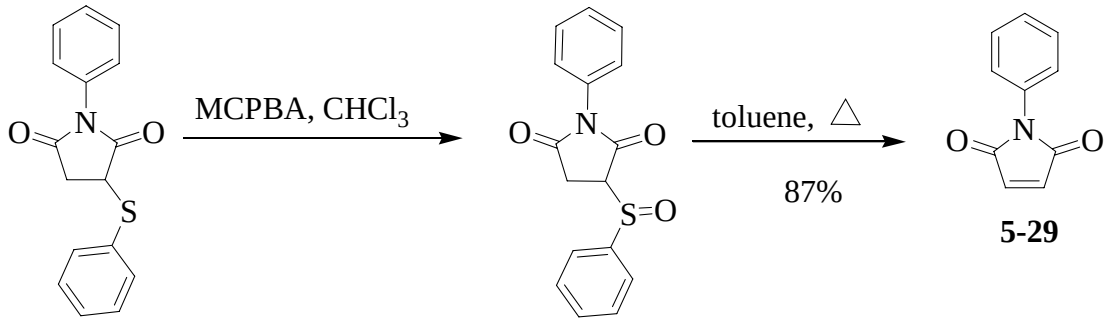
5-32 **5-33**

87%

Scheme 5-11. Synthesis of succinimide **5-33**

The ability to regenerate phenyl maleimide **5-29** after the nucleophilic addition of the thiol was an essential part in the design of the MIP. Since Michael-type additions are reversible by heating, we attempted to reform the phenyl maleimide by this method.[28] Succinimides **5-31** and **5-33** were refluxed in $CHCl_3$ (b.p. = 62 °C) or toluene (b.p. = 111 °C) for 36 hr and monitored by TLC to obtain phenyl maleimide **5-29**. Even with toluene's high boiling point, refluxing only provided the starting material succinimides. Bases can also be employed with heating to favor the reversible Michael addition.[28] Therefore, we refluxed succinimides **5-31** and **5-33** in THF, $CHCl_3$, and toluene with base (triethylamine and *t*-butoxide) to obtain phenyl maleimide **5-29**, but only the starting materials were present. Even though Michael additions are known to be reversible, thiol anions are very good nucelophiles and poor leaving groups, therefore favoring the succinimides.[28]

The thiol needed to be converted to a more reactive leaving group. For example, sulfonate esters, such as mesilates, tosylates, and triflates, are excellent leaving groups because the resulting sulfonate ion is a weak base.[28] Thus, we envisioned the oxidation of **5-31** to obtain sulfoxide **5-34**. Treatment with heat would eliminate the sulfoxide and regenerate the maleimide. Utilizing typical oxidation procedures,[134] a solution of *m*-chloroperbenzoic acid (MCPBA), **5-31**, and $CHCl_3$ were stirred at room temperature for 1 hr to obtain sulfoxide **5-34** (Scheme 5-12). The compound was refluxed in toluene for 1 hr. to give the elimination product **5-29** in high yields. However, for the proposed MIP system, MCPBA could potentially oxidize the fluorescein molecule or form epoxides from the alkenes of the polymer backbone. The solution was to hydrogenate the polymer double bonds prior to oxidation. The MIP system would be made by a series of experiments in this order: 1) add the thiol to the fluoresceinyl maleimide; 2) couple the dye with the norbornene moiety to allow ROMP; 3) hydrogenate the olefins on the polymer backbone to prevent epoxidation; 4) and oxidized and eliminate the thiol to regenerate the maleimide.



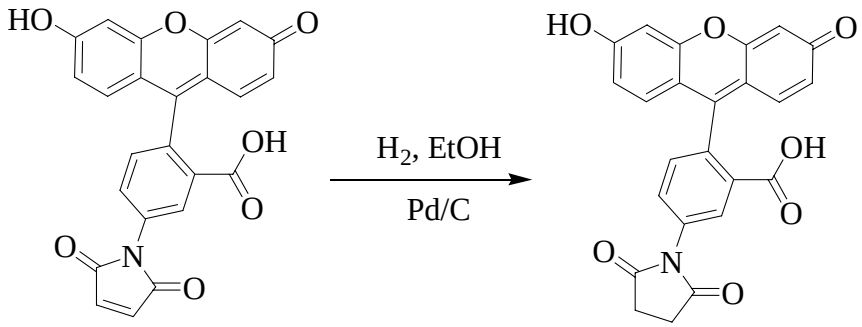
5-31 **5-34** **5-29**

Scheme 5-12. Oxidation and elimination reactions

A quick test was performed to ensure hydrogenation conditions would not reduce the aromatic rings on the fluoresceinyl dye. One of the most common methods for

hydrogenation of olefins involves the use of H_2 and palladium on activated carbon (Pd/C) as the catalyst. Palladium is usually preferred as the catalyst for the reduction of double bonds in molecules possessing several functional groups, such as esters[135]. Based on previous studies in our lab, hydrogenation of olefins can usually proceed in less than one hour at atmospheric pressure.[88] We wanted to employ these reaction conditions to fluorescein maleimide **5-7** as a model, where we expect to observe the hydrogenation of the maleimide but not the reduction of the double bonds of the aromatic rings (Scheme 5-13). For the proposed MIP, hydrogenation of the maleimide would not be an issue since thiol **5-5** would have been added to the double bond of the maleimide at this point.

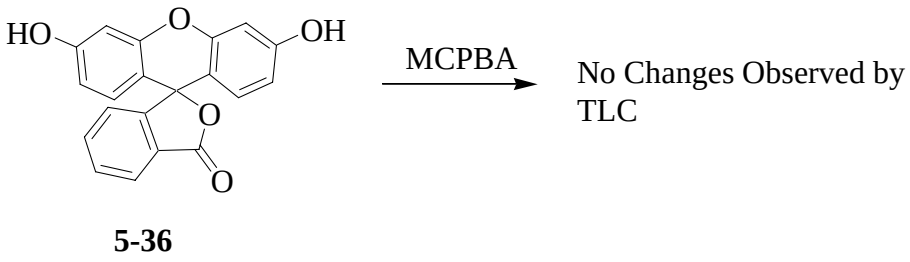
Pd/C was introduced to a solution of fluorescein maleimide **5-7** and ethanol. After evacuating the headspace, hydrogen gas was admitted via a balloon that was attached to the reaction flask by a three way stopcock. The reaction was stirred for a total of 45 min. Observation of the reaction by thin layer chromatography (TLC) indicated the formation of a new product with small amounts of starting material present. Hydrogenation for another 30 min revealed no major changes by TLC. After removal of the solvent and the catalyst, NMR analysis of the crude product indicated selective hydrogenation of the maleimide alkene but the aromatic rings of the molecule were not affected.



5-7 **5-35**

Scheme 5-13: Hydrogenation of fluorescein maleimide **5-7**.

We then examined the affects of MCPBA on fluorescein **5-36**, which is commercially available and less expensive then fluorescein maleimide **5-7** (Scheme 5-14). Unlike succinimide **5-31**, which is soluble in $CHCl_3$, fluorescein **5-36** is a very polar compound. We conducted the oxidation experiments in EtOH or in an aqueous solution of $NaHCO_3$ (0.3 N) based on literature procedures.[136] The reactions were monitored by TLC, and after 1.5 hr, we observed mostly starting material present and no decomposition of the fluorescein **5-36**.



5-36

Scheme 5-14: Attempted oxidation of fluorescein **5-36**

5.3 Conclusions

To examine the viability of developing a nerve gas detector using ROMP in molecular imprinting technology, several model studies were conducted. We first investigated the Michael addition of thiol compounds to the maleimide. Then several reaction conditions were tested to eliminate the thiol and regenerate the maleimide. The oxidation/elimination reaction using MCPBA and heat appeared to be most reasonable, although hydrogenation of the polymer will be required to eliminate side reactions. We were able to demonstrate the synthesis of fluorescein maleimide and the coupling with 5-norbornene-2-carboxylic acid, which is required for the ROMP. Several more experiments are required, such as analyzing the fluorescent properties of fluorescein type molecules, before we can synthesize the MIP. However, based on preliminary studies, it

seems promising that a MIP could be generated to detect thiol compounds using fluorogenic maleimide.

CHAPTER 6
EXPERIMENTALS

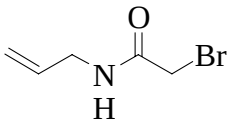
6.1 General Method and Instrumentation

All moisture and air-sensitive reactions were performed under an atmosphere of argon with flame-dried glassware. Solvents were distilled under N_2 from appropriate drying agents according to established procedures. Analytical Thin Layer Chromatography (TLC) was performed on Kiesel gel 20 F-254 pre-coated 0.25mm silica gel plates. UV light, phosphomolybdic acid in ethanol, anisaldehyde in ethanol, permanganate, and vanillin were used as indicators. Column flash chromatography was carried out using silica gel 60 (40-60 μm). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Gemini, VXR, or Mercury 300 MHz spectrometer. Carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 75 MHz on the same spectrometers. Chemical shifts were reported in ppm downfield relative to tetramethylsilane (TMS) as an internal standard. The *J*-values are in hertz. The following abbreviations are used: s, singlet; d, doublet; t, triplet, m, multiplet. Infrared spectra were recorded using a Bruker Vector 22 IR and reported in wavenumbers (cm^{-1}). High-resolution mass spectroscopy (HRMS) was performed by the Mass Spectroscopy Laboratory at the University of Florida. Electron ionization (EI) was carried out on a Finnigan MAT 95Q hybrid-sector mass spectrometer at 70 eV using a direct insertion probe. Chemical Ionization (CI) was carried out at 150 eV using a direct insertion probe in the presence of methane. Electrospray ionization (ESI) was performed on a Finnigan LCQ – Quadrupole Ion Trap. Elemental analyses were performed by the Elemental

Analysis Service at University of Florida, Department of Chemistry. High Performance Liquid Chromatography (HPLC) analyses were performed on a Shimadzu LC-6AD. Yields reported refer to the isolated materials unless otherwise indicated. Melting points were determined with a Barnstead or Thomas Hoover melting point apparatus and are uncorrected.

6.2 Experimental Procedures and Data

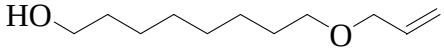
N-Allyl-2-bromo-acetamide **(2-9)**



Following procedures similar to literature[86], a flame dried 250 mL round bottom flask was charged with bromoacetyl bromide **2-14** (8.0 mL, 61 mmol) and freshly distilled CH_2Cl_2 (80 mL) and cooled in an ice bath to 0 °C. To the stirred solution was added a solution of allylamine **2-13** (12 mL, 160 mmol) and CH_2Cl_2 (40 mL) drop-wise and the mixture was stirred at 0°C for 4 h. Distilled H_2O was added and the organic layer was extracted and washed with 1 *N* HCl, followed by H_2O. After drying (anhydrous Na_2SO_4), concentration gave crude **2-9-** as a yellow-orange oil. Recrystallization from hexane and ethyl ether (9:1) at 0 °C overnight gave **2-9** (8.0 g, 73%) as analytically pure white crystals, m.p. 27°C.

2-9: R_f = 0.39 (hexane/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 6.62 (s, 1H), 5.85 (ddt, J = 17.2, 10.2, 5.3 Hz, 1H), 5.23 (dq, J = 17.1, 1.5 Hz, 1H), 5.19 (dq, J = 10.0, 1.5 Hz, 1H), 3.96-3.90 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 165.86, 133.33, 116.82, 42.48, 29.12; IR (neat) 3285, 3082, 1654, 1552, 1430, 1309, 1211, 1132, 990, 925 cm^{-1}; HRMS (CI pos) for C_5H_9BrNO $[M+H]^+$: calcd 177.9868, found 177.9862.

8-Allyloxy-octan-1-ol **(2-10)**



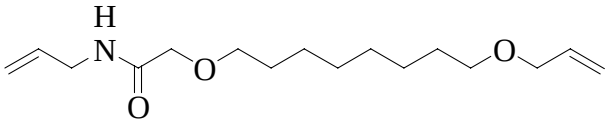
A flame dried flask flushed with argon was charged with sodium hydride (60% mass) (1.1 g, 28 mmol). The gray powder was washed three times with pentane to remove the protective oil. To the NaH was added 1,8-octanediol (**2-11**) (3.55 g, 24.3 mmol) dissolved in THF (25 mL). Tetrabutylammonium iodide (TBAI) (0.0251 g, 0.0680 mmol) was added and the mixture stirred for 40 min. A solution of allyl bromide (1.9 mL, 22 mmol) and THF (25 mL) was added drop-wise to the reaction flask and heated at reflux for 12 h. The reaction mixture was cooled to room temperature, neutralized with saturated ammonium chloride and extracted 3x with EtOAc. The combined organic layers were washed with brine, dried with sodium sulfate (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel with CH_2Cl_2/MeOH (100:0-98:2) to give colorless oil **2-10** (2.9 g, 70%) and double Williamson etherification product **2-12** (0.62 g, 25%).

2-10: R_f = 0.23 (CH_2Cl_2/MeOH, 96:4); ^{1}H NMR ($CDCl_3$) δ 5.91 (ddt, J = 17.1, 10.2, 5.6 Hz, 1H), 5.26 (dq, J = 17.2, 1.6 Hz, 1H), 5.16 (dq, J = 10.7, 1.4 Hz, 1H), 3.95 (dt, J = 5.6, 1.3 Hz, 2H), 3.61 (t, J = 6.5 Hz, 2H), 3.41 (t, J = 6.7 Hz, 2H), 1.66 (s, 1H), 1.60-1.49 (m, 4H), 1.31 (s, 8H); ^{13}C NMR ($CDCl_3$) δ 135.20, 116.93, 71.97, 70.63, 63.10, 32.91, 29.88, 29.60, 29.52, 26.26, 25.84; IR (neat) 3385 (br), 2931, 2856, 1647, 1463, 1348, 1101 cm^{-1}; HRMS (CI pos) for $C_{11}H_{23}O_2$ $[M+H]^+$: calcd 187.1698, found 187.1697.

2-12: R_f = 0.74 (CH_2Cl_2/MeOH, 96:4); ^{1}H NMR ($CDCl_3$); 5.90 (ddt, J = 17.2, 10.3, 5.65 Hz, 1H), 5.89 (ddt, J = 17.2, 10.3, 5.65, 1H), 5.25 (dq, J = 17.1, 1.7 Hz 1H), 5.24 (dq, J = 17.1, 1.8, 1H), 5.15 (dq, J = 10.4, 1.5 Hz, 1H), 5.14 (dq, J = 10.4, 1.5 Hz, 1H), 3.94 (dt, J = 5.6, 1.5, 2H), 3.93 (dt, J = 5.4, 1.5 Hz, 2H), 3.40 (t, J = 6.7 Hz, 2H), 3.39 (t, J

= 6.7 Hz, 2H), 1.61-1.50 (m, 4H), 1.30 (s, 8H); ^{13}C NMR ($CDCl_3$) δ 135.20, 116.81, 71.93, 70.59, 29.88, 29.56, 26.26; IR (neat) 3080, 2932, 2856, 1647, 1457, 1420, 1400, 1347, 1264, 1106 cm^{-1}; HRMS (CI pos) for $C_{14}H_{27}O_2$ $[M+H]^+$: calcd 227.2011, found 227.2015.

N-Allyl-2-(8-allyloxy-octyloxy)-acetamide **(2-15)**

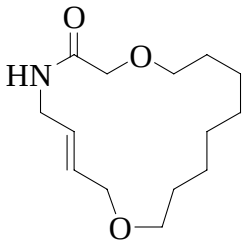
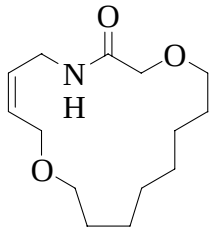


A flame dried 25 mL round bottom flask under argon was charged with alcohol **2-10** (2.97 g, 16.0 mmol) and THF (9.5 mL). NaH (60% mass, 0.64 g, 16 mmol) was added portion wise slowly into the stirred solution and the mixture continued to stir for 20 min. TBAI (0.0259 mg, 0.0701 mmol) and allyl bromo acetamide **2-9** (2.20 g, 12.3 mmol) were added and the mixture was heated at reflux for 4 h. The mixture was diluted with CH_2Cl_2 and washed with brine. The organic layer was dried with anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude oil was purified by chromatography on silica gel with hexane/EtOAc (100:0-80:20) to give **2-15** (1.9 g, 54%) as a clear oil.

2-15: R_f = 0.53 (hexane/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 6.63 (s, 1H), 5.87 (ddt, J = 17.2, 10.7, 5.4 Hz, 1H), 5.82 (ddt, J = 17.0, 10.6, 5.3 Hz, 1H), 5.23 (dq, J = 17.2, 1.6 Hz, 2H), 5.12 (dq, J = 10.5, 1.6 Hz, 2H), 3.94-3.86 (m, 6H), 3.46 (t, J = 6.6 Hz, 2H), 3.38 (t, J = 6.7 Hz, 2H), 1.61-1.49 (m, 4H), 1.28 (s, 8H); ^{13}C NMR ($CDCl_3$) δ 169.99, 135.31, 134.24, 116.93, 116.61, 72.12, 72.04, 70.67, 70.42, 41.34, 29.98, 29.71, 29.63, 29.56, 26.36, 26.22; IR (neat) 3426, 3335, 3081, 2932, 2856, 1738, 1676, 1526, 1447, 1432, 1342, 1277, 1111, 997, 921 cm^{-1}; HRMS (CI pos) for $C_{16}H_{30}O_3N$ $[M+H]^+$: calcd 284.2226, found 284.2225.

6.2.1 General procedure for ring-closing metathesis

Trans-cycloheptadecene **2-16** and *Cis*-cycloheptadecene **2-17**

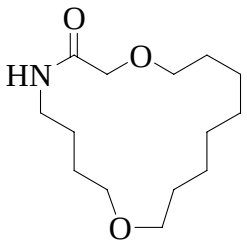
To a stirred solution of diene **2-15** (377 mg, 1.33 mmol) and CH_2Cl_2 (750 mL) was added a few crystals of butylated hydroxytoluene (BHT). A solution of Grubbs' 2nd generation catalyst **1-4** (108 mg, 0.127 mmol) and CH_2Cl_2 (100 mL) was added and heated at reflux for 16 h. The reaction was cooled to room temperature, quenched with ethyl vinyl ether (*ca.* 3 mL), and maintained for 1 hour. The solution was concentrated under reduced pressure and the residue chromatographed on silica gel with hexane/EtOAc (9:1-7:3) to give the *E* isomer **2-16** (200 mg, 58%) and *Z* isomer **2-17** (8.2 mg, 2.4%) as a brown oil.

2-16: R_f = 0.35 (EtOAc/hexane, 4:1); ^{1}H NMR ($CDCl_3$) δ 6.59 (s, 1H), 5.80 (dd, J = 14.8, 4.4 Hz, 1H), 5.72 (dd, J = 14.8, 4.3 Hz, 1H), 3.99-3.89 (m, 6H), 3.53 (t, J = 5.7 Hz, 2H), 3.46 (t, J = 5.7 Hz, 2H), 1.65-1.29 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 169.95, 130.11, 127.92, 71.92, 70.25, 69.91, 69.33, 39.95, 29.18, 28.91, 28.39, 28.10, 26.08, 25.27; IR (neat) 3419, 2929, 2856, 1683, 1525, 1460, 1340, 1263, 1111 cm^{-1}; HRMS (CI pos) for $C_{14}H_{26}NO_3$ $[M+H]^+$, calcd 256.1913, found 256.1911.

2-17: 0.43 (EtOAc/hexane, 4:1); ^{1}H NMR ($CDCl_3$) δ 6.65 (s, 1H), 5.89 (dd, J = 10.6, 6.3 Hz, 1H), 5.81 (dd, J = 10.3, 7.2 Hz, 1H), 4.03-3.90 (m, 6H), 3.55-3.45 (m, 4H), 1.70-1.23 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 169.95, 130.30, 130.01, 71.25, 70.67, 70.38,

65.63, 35.42, 29.25, 28.45, 27.27, 27.16, 24.91, 24.61; HRMS (ESI-FTICR) for $[2M+Na]^+$, calcd 533.3561, found 533.3580.

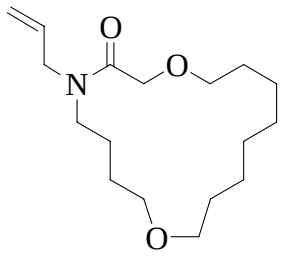
Unsaturated Lactam **2-18**



Palladium on activated carbon (10% Pd) (58 mg, 0.055 mmol) was added to a solution of **2-16** (507 mg, 1.99 mmol) and EtOAc (9 mL). The headspace was evacuated using a three way stop cock attached to a water aspirator. Hydrogen was admitted via a balloon also attached to the stop cock. The reaction mixture was stirred for 45 min and the catalyst removed by filtering through a small pipette column of Celite. The column was rinsed with EtOAc (3 x 5 mL) and the combined fractions concentrated under reduced pressure leaving crude **2-18** (500 mg, 98%) as a yellow-brown oil. The hydrogenated product was used in the next step without further purification.

2-18: R_f = 0.32 (EtOAc/hexane, 4:1); ^{1}H NMR ($CDCl_3$) δ 6.63 (s, 1H), 3.83 (s, 2H), 3.43-3.21 (m, 8H), 1.66-1.21 (m, 16H); ^{13}C NMR ($CDCl_3$) δ 170.00, 71.61, 70.23, 70.12, 38.05, 28.97, 28.85, 27.54, 27.38, 26.70, 26.61, 25.13, 24.70; IR (neat) 3420, 2932, 2857, 1681, 1530, 1446, 1340, 1261, 1120 cm^{-1}; HRMS (CI pos) for $C_{14}H_{28}NO_3$ $[M+H]^+$: calcd 258.2069, found 258.2073.

Lactam **2-8**



To a stirred solution of **2-18** (570 mg, 2.22 mmol) in THF (2.2 mL) under argon was added NaH (60% mass, 270 mg, 6.7 mmol) slowly in portions. The mixture was stirred for 30 min. TBAI (8.18 mg, 0.0222 mmol) and allyl bromide (0.97 mL, 11 mmol) were added and the reaction was heated at reflux for 9 h. The reaction mixture was cooled to room temperature, neutralized with saturated ammonium chloride, and extracted with EtOAc. The organic layer was washed with saturated NaCl, dried with $MgSO_4$, and concentrated under reduced pressure. Purification by chromatography on silica gel with hexane/EtOAc (4:1) gave **2-8** (0.41 g, 62%) as a slightly yellow oil.

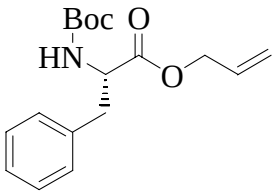
2-8: R_f = 0.37 (hexane/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 5.84-5.68 (m, 1H), 5.18-5.07 (m, 2H), 4.17-3.93 (m, 4H), 3.57-3.22 (m, 8H); 1.79-1.21 (m, 16H); ^{13}C NMR ($CDCl_3$) δ 169.89, 169.26, 133.49, 133.39, 117.23, 116.29, 71.67, 71.45, 71.41, 70.83, 70.21, 70.15, 70.03, 70.00, 48.97, 47.95, 47.32, 44.95, 29.51, 28.71, 28.67, 28.47, 28.03, 27.53, 27.35, 27.11, 26.71, 26.58, 25.87, 25.71, 25.08, 24.26, 24.22; IR (neat) 2931, 2858, 1651, 1459, 1352, 1282, 1232, 1118, 1038, 995, 922 cm^{-1}; HRMS (CI pos) for $C_{17}H_{32}NO_3$ $[M+H]^+$: calcd 298.2382, found 298.2391. Anal. calcd for $C_{17}H_{31}NO_3$: C, 68.65; H, 10.51; N, 4.71. Found: C, 68.31; H, 10.73; N, 4.94%

6.2.2 General amino acid coupling procedures

To a cooled (0 ˚C) solution of amino acid (7.7 mmol) and CH_2Cl_2 (13 mL) was added 1,3-diisopropylcarbodiimide (DIC) (15 mmol), 4-(dimethylamino)pyridine

(DMAP) (1.5 mmol), and hydroxybenzotriazole (HOBt) (8.0 mmol). After stirring the mixture for 5 min, the alcohol (12 mmol) was slowly added. The mixture was allowed to warm to room temperature while stirring for a total of 3 h. All solids were filtered and the filtrate was concentrated under reduced pressure. The crude product was purified on a silica gel column, eluting with hexane:EtOAc to provide the amino acid derivative.

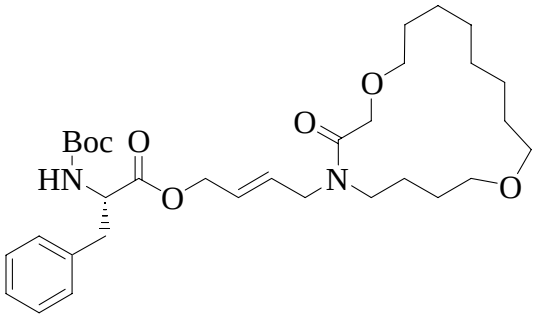
t-Boc-allyl ester of phenylalanine **2-7**



Following amino acid coupling procedures, t-Boc-L-phenylalanine (2.04 g, 7.69 mmol), CH_2Cl_2 (13 mL), DIC (2.4 mL, 15 mmol), DMAP (0.186 g, 1.52 mmol), HOBt (1.08 g, 7.99 mmol), and allyl alcohol (0.85 mL, 12 mmol) gave **2-7** (2.2 g, 92%) as a white solid, m.p. 71-72 ◦C.

2-7: R_f = 0.35 (hexane/EtOAc, 4:1); $[\alpha]^{25}_D$ = -8.05° (c = 1.1 , MeOH); 1H NMR ($CDCl_3$) δ 7.08-7.32 (m, 5H), 5.84 (ddt, J = 17.2, 10.4, 5.2 Hz, 1H), 5.28 (dq, J = 17.1, 1.4 Hz, 1H), 5.22 (dq, J = 10.3, 1.3 Hz, 1H), 4.98 (d, J = 7.9 Hz, 1H), 4.63-4.54 (m, 3H), 3.11 (dd, J = 13.8, 6.3 Hz, 1H), 3.04 (dd, J = 13.8, 6.5 Hz, 1H), 1.40 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.63, 155.14, 136.07, 131.59, 129.42, 128.58, 127.06, 118.94, 79.91, 65.97, 54.57, 38.39, 28.39; IR (neat) 3362, 3088, 2971, 1705, 1509, 1455, 1368, 1169, 1053; HRMS (CI pos) for $C_{17}H_{24}NO_4$ $[M+H]^+$: calcd 306.1705, found 306.1703. Anal. calcd for $C_{17}H_{23}NO_4$: C, 66.86; H, 7.59; N, 4.59. Found: C, 66.65, H, 7.78; N, 4.52%. Spectral data are in agreement with literature. Lit[100] $[\alpha]^{29}_D$ = -10.2◦ (c = 1.10 , MeOH).

Cross-metathesis product **2-1**

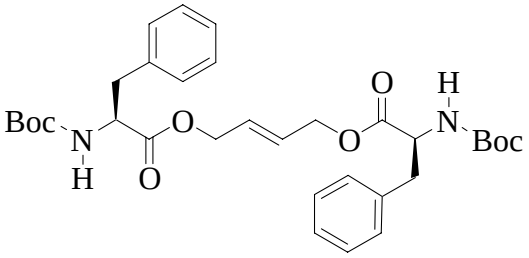


To a stirred solution of **2-8** (140 mg, 0.469 mmol), **2-7** (290 mg, 0.950 mmol), and $CHCl_3$ (0.50 mL), was added a solution of catalyst **1-4** (19.9 mg, 0.0234 mmol) and $CHCl_3$ (0.40 mL). The solution was heated at reflux for 21 h while flushing the headspace with argon to remove evolved ethylene. The reaction was allowed to cool to room temperature and quenched with ethyl vinyl ether (EVE, *ca*. 0.75 mL). The solution was stirred for 30 min and concentrated under reduced pressure. Purification by chromatography with hexane/EtOAc (90:10-65:35) gave cross-metathesis product **2-6** (130 mg, 48%) as a colorless oil and homodimer **2-19** (120 mg, 45%) as a white solid, m.p. = 148-150 °C. Starting materials **2-8** (40 mg, 29%) and **2-7** (71 mg, 25%) were recovered as well.

2-6: R_f = 0.26 (hexane/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 7.09-7.32 (m, 5H), 5.79-5.53 (m, 2H), 4.99 (d, J = 8.5 Hz, 1H), 4.62-4.53 (m, 3H), 4.19-3.94 (m, 4H), 3.59-3.22 (m, 8H); 3.11 (dd, J = 13.4, 6.3 Hz, 1H), 3.03 (dd, J = 13.4, 6.5 Hz, 1H); 1.81-1.14 (m, 25H); ^{13}C NMR ($CDCl_3$) δ 171.75, 169.78, 169.38, 155.19, 136.07, 130.67, 130.50, 129.47, 129.43, 128.66, 127.15, 126.16, 125.36, 80.02, 71.80-70.04 (7 lines), 65.20-64.82 (2 lines), 54.55, 47.72-44.97 (4 lines), 38.46, 29.54-24.26 (13 lines); IR (neat) 3439, 2933, 2860, 2247, 1712, 1640, 1497, 1456, 1367, 1254, 1168, 1114, 910, 733 cm^{-1}; $[\alpha]^{25}_D$ = +8.7° (c = 1, $CHCl_3$); HRMS (CI pos) for $C_{32}H_{51}N_2O_7$ $[M+H]^+$: calcd 575.3696, found

575.3680. Anal. calcd for $C_{32}H_{50}N_2O_7$: C, 66.87; H, 8.77; N, 4.87. Found: C, 66.56; H, 9.00; N, 4.70%.

Homodimer Boc-allyl ester phenylalanine **2-19**



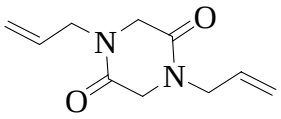
2-19: R_f = 0.67 (hexane/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 7.34-7.09 (m, 10H), 5.76-5.64 (m, 2H), 4.99 (d, J = 7.9 Hz, 2H), 4.65-4.54 (m, 6H), 3.10 (dd, J = 13.6, 5.8 Hz, 2H), 3.03 (dd, J = 13.6, 5.8 Hz, 2H), 1.41 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 171.76, 155.25, 136.07, 129.53, 128.76, 128.06, 127.26, 80.18, 64.70, 54.64, 38.54, 28.47; IR (neat) 3367, 2977, 1715, 1498, 1455, 1367, 1252, 1166, 1054, 1022 cm-1; HRMS (ESI-FTICR) for $[M+Na]^+$: calcd 605.2833, found 605.2859. Anal. calcd for $C_{32}H_{42}N_2O_8$: C, 65.96; H, 7.27; N, 4.81. Found: C, 66.16, H, 7.53; N, 4.77%.

6.2.3 General procedure for ethylenolysis of **2-6**

A flame dried 5 mL round bottom flask equipped with a reflux condenser capped with a three-way stopcock was charged with **2-6** (110 mg, 0.191 mmol) and CH_2Cl_2 (95 µl). To the stirred solution was added a solution of catalyst **1-4** (3.5 mg, 0.0041 mmol) in CH_2Cl_2 (95 µl). The headspace was evacuated using a water aspirator attached to the stopcock. Ethylene gas, used without purification, was admitted via a balloon also attached to the stopcock. The solution was heated at reflux for 2.5 h, cooled to room temperature, and quenched with EVE (*ca*. 0.5 mL). Concentration by reduced pressure and purification of the residue by chromatography on silica gel with hexane/EtOAc

(10:0-8:2) gave **2-8** (20 mg, 36%) and **2-7** (22 mg, 37%) as a colorless oil. Starting material **2-1** (30 mg, 27%) and amino acid dimer **2-19** (2.2 mg, 4%) were isolated as well. All spectral and TLC data were identical to data for **2-7**, **2-6**, **2-8**, and **2-19** previously isolated.

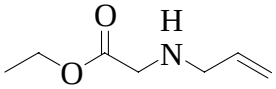
N-allyl dimer scaffold **3-3**



NaH (60% mass, 4.0 g, 100 mmol) was added in portions to a stirred solution of glycine anhydride (**3-10**) (3.14 g, 27.5 mmol) and anhydrous DMF (55 mL). After stirring for an additional 15 minutes, TBAI (1.60 g, 4.33 mmol) and allyl bromide (12 mL, 140 mmol) were added. The reaction mixture was heated maintained at room temperature for 3.5 h, then quenched with H_2O. DMF and H_2O were removed *in vacuo*, leaving behind an orange semi-solid. EtOAc was added to the residue and the sodium salts filtered. The filtrate was concentrated *in vacuo*. Column chromatography on silica gel with EtOAc/Hexane (7:3) afforded **3-3** (4.1 g, 77%) as a white solid, m.p. 97.5-99.0 $^{\circ}$C.

3-3: R_f = 0.24 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 5.82-5.63 (ddt, J = 17.1, 10.2, 6.3 Hz, 2H), 5.29-5.17 (m, 4H), 4.02-3.97 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 163.36, 130.94, 119.80, 49.27, 48.32; IR (KBr) 3079, 2914, 1656, 1487, 1441, 1415, 1336, 1294, 1193, 1142, 1074, 1011 cm^{-1}; HRMS (EI pos) for $C_{10}H_{14}N_2O_2$ $[M]^+$: calcd 194.1055, found 194.1047. Anal. calcd for $C_{10}H_{14}N_2O_2$: C, 61.84; H, 7.27; N, 14.42. Found C, 61.75; H, 7.42; N, 14.29%.

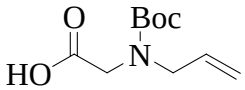
H-(Nall)Gly-OEt **3-13**



Following a procedure reported in the literature,[47] ethyl bromoacetate (**3-12**) (11 mL, 99 mmol) in THF (160 mL) was added drop-wise to a cooled solution of allylamine (**3-11**) (16 mL, 210 mmol) in THF (260 mL). The solution was maintained at 0 °C for 2 h, concentrated *in vacuo*, and suspended in Et_2O. The suspension was filtered through a coarse filtration frit and washed with Et_2O. The filtrate was concentrated *in vacuo*. Column chromatography on silica gel with Et_2O afforded ester **3-13** (8.6 g, 60%) as an oil.

3-13: R_f = 0.29 (Et_2O); ^{1}H NMR ($CDCl_3$) δ 5.83 (ddt, J = 17.3, 10.4, 6.0 Hz, 1H), 5.16 (dd, J = 17.4, 1.5 Hz, 1H), 5.08 (dd, J = 10.4, 1.3 Hz, 1H), 4.16 (q, J = 7.3 Hz, 2H), 3.36 (s, 2H), 3.23 (d, J = 6.1, 2H), 1.75 (s, 1H), 1.24 (t, J = 7.2, 3H); ^{13}C NMR ($CDCl_3$) δ 172.58, 136.21, 116.70, 60.85, 51.94, 50.08, 14.34. All spectral data are in agreement with literature.[47]

t-Boc-(Nall)Gly-OH **3-14**



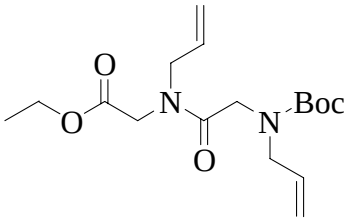
Boc_2O (2.33 g, 10.7 mmol) was added to a stirred solution of ester **3-13** (1.38 g, 9.65 mmol) in THF (40 mL). The solution was maintained for 5 h. The solvent was removed in vacuo to give the t-Boc-protected ester as an oil; R_f = 0.44 (Et_2O/hexane, 2:3). The oil was redissolved in MeOH (12 mL), followed by the addition of 4 *N* NaOH (3 mL). After stirring for 3 h, MeOH was removed *in vacuo*. Water (60 mL) was added and the aqueous layer washed with Et_2O (2 x 50 mL). The aqueous layer was acidified with 1 *N* HCl (12 mL) and extracted with EtOAc (3 x 70 mL). The combined organic

layers were washed with brine, dried with Na_2SO_4, and concentrated in vacuo to give t-Boc-protected N-allyglycine **3-14** (2.1 g, quantitative yields) as a colorless oil. Compound **3-14** was used in the next step without further purification.

3-14: ^{1}H NMR ($CDCl_3$) δ 10.80 (s, 1H), 5.84-5.65 (m, 1H), 5.21-5.03 (m, 2H), 4.02-3.84 (m, 4H), 1.43 and 1.40 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 175.09, 174.98, 155.92, 155.33, 133.48, 133.30, 117.95, 117.19, 80.94, 50.87, 50.31, 47.71, 47.55, 28.32, 28.26 [2 rotamers detected]. All spectral data are in agreement with literature.[47]

6.2.4 General procedure for EDCI coupling

t-Boc-(Nall)Gly-(Nall)Gly-OEt **3-15**

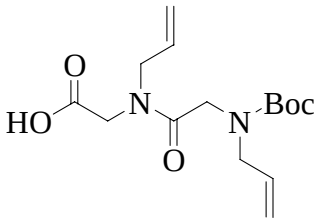


A flame dried flask under argon was charged with acid **3-14** (4.26 g, 19.8 mmol), EDCI (5.70 g, 29.7 mmol), HOBt (4.02 g, 29.8 mmol), DMAP (187 mg, 1.53 mmol), DIPEA (10 mL, 57 mmol), and CH_2Cl_2 (157 mL). Ester **3-13** (2.70 g, 18.9 mmol), was added to the stirred solution and maintained for 18 h. The solvent was removed *in vacuo*, and the residue redissolved in EtOAc (290 mL). The organic layer was washed with 1 *N* $KHSO_4$ (3 x 150 mL), 1 *N* $NaHCO_3$ (3 x 150 mL), water (150 mL), and brine (150 mL). The organic layer was then dried with Na_2SO_4 and concentrated *in vacuo* to give **3-15** (6.4 g, quantitative yields) as a viscous colorless oil. The oil was used in the next reaction without further purification.

3-15: R_f = 0.39 (hexane/EtOAc, 1:1). ^{1}H NMR ($CDCl_3$) δ 5.85-5.63 (m, 2H), 5.26-5.02 (m, 4H), 4.21-3.82 (m, 10H), 1.41 (s, 9H), 1.23 (t, J = 6.8 Hz, 3H). Spectral data are in agreement with literature.[47]

6.2.5 General hydrolysis procedures

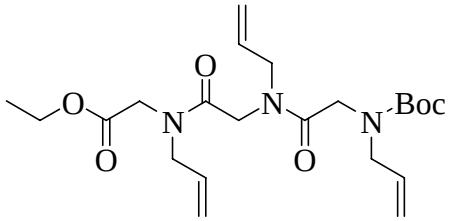
t-Boc-(Nall)Gly-(Nall)Gly-OH **3-16**



4 *N* NaOH (8.6 mL) was added to a stirred solution of ester **3-15** (6.38 g, 18.7 mmol), and MeOH (23 mL). After 3 h, MeOH was removed in vacuo. Water (100 mL) was added and the aqueous layer washed with Et_2O (2 x 90 mL). The aqueous layer was acidified with 1 *N* HCl (35 mL) and extracted with EtOAc (3 x 110 mL). The combined organic layers were washed with brine, dried with Na_2SO_4, and concentrated in vacuo to give **3-16** (5.8 g, 98%) as a viscous slightly yellow oil. Acid **3-16** was used in the next step without further purification.

3-16: ^{1}H NMR ($CDCl_3$) δ 5.85-5.64 (m, 2H), 5.22-5.08 (m, 4H), 4.10-3.85(m, 8H), 1.44 and 1.43 (s, 9H).

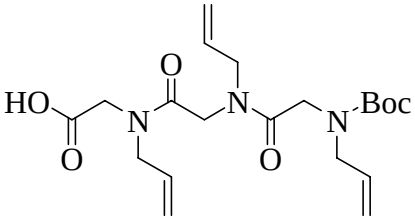
t-Boc-(*N*all)Gly-(*N*all)Gly-(*N*all)Gly-OEt **3-17**



Following general EDCI coupling procedures, acid **3-16** (1.19 g, 3.82 mmol), ester **3-13** (502 mg, 3.50 mmol), EDCI (1.00 g, 5.22 mmol), HOBt (713 mg, 5.28 mmol), DMAP (40 mg, 0.327 mmol), DIPEA (2.0 mL, 11.5 mmol), and CH_2Cl_2 (29 mL) yielded ester **3-17** (1.52 g, quantitative yields) as a viscous slightly yellow oil. The oil was used in the next step without further purification.

3-17: R_f = 0.33 (hexane/EtOAc, 1:2); ^{1}H NMR ($CDCl_3$) δ 5.82–5.55 (m, 3H), 5.24-4.98 (m, 6H), 4.21-3.78 (m, 14H), 1.36 and 1.34 (s, 9H), 1.18 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 169.27-168.67 (4 lines), 155.67-155.41 (2 lines), 133.93-131.96 (7 lines), 118.50-116.43 (5 lines), 79.94-79.88 (2 lines), 61.67-61.16 (3 lines), 50.84-49.61 (5 lines), 48.30-46.35 (7 lines), 28.29, 14.12; IR (neat) 3082, 2980, 2934, 1747, 1675, 1463, 1367, 1250, 1194 cm^{-1}; HRMS (ESI-FTICR) for $[M+Na]^+$: calcd 460.2418, found 460.2414.

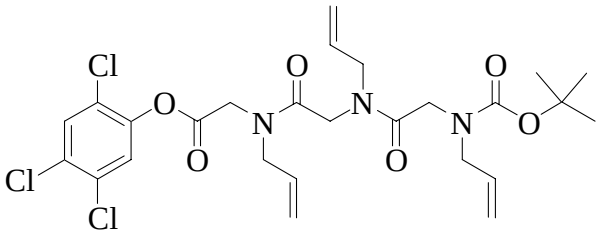
t-Boc-(Nall)Gly-(Nall)Gly-(Nall)Gly-OEt **3-18**



Following general hydrolysis procedures, ester **3-17** (6.38 g, 18.7 mmol), 4 *N* NaOH (8.7 mL), MeOH (23 mL), and 1 *N* HCl (35 mL) yielded **3-18** (5.8 g, quantitative yields) as a viscous slightly yellow oil. Acid **3-18** was used in the next step without further purification.

3-18: ^{1}H NMR ($CDCl_3$) δ 10.56 (s, 1H), 5.89-5.57 (m, 3H), 5.31-4.97 (m, 6H), 4.30-3.68 (m, 12H), 1.39 and 1.37 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 171.56-168.80 (8 lines), 156.14-155.57 (3 lines), 133.77-131.87 (7 lines), 118.67-116.75 (5 lines), 80.60-80.48 (2 lines), 50.86-49.82 (6 lines), 47.96-46.74 (4 lines), 28.36; IR (neat) 3083, 2980, 2934 (region broader than ester **3-17**), 1738, 1672, 1477, 1407, 1367, 1250, 1173 cm^{-1}; HRMS (ESI-FTICR) for $[M+Na]^+$: calcd 432.2105, found 432.2120.

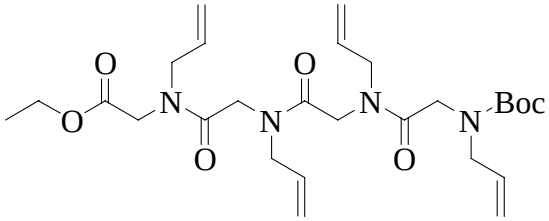
t-Boc protected 2,4,5-trichlorophenol ester **3-20**



Following general EDCI coupling procedures, acid 4-14 (4.71g, 11.5 mmol), 2,4,5-trichlorophenol (**3-19**) (2.06 g, 10.5 mmol), EDCI (3.06 g, 16.0 mmol), HOBt (2.12 g, 15.7 mmol), DMAP (0.130 g, 1.06 mmol), DIPEA (5.5 mL, 32 mmol), and CH_2Cl_2 (87 mL) yielded ester **3-20** (3.9 g, 58%) as a white solid, m.p. = 81.5-82.5 °C.

3-20: R_f = 0.34 (Et_2O/hexane, 9:1); ^1H NMR ($CDCl_3$) δ 7.54 (s, 1H), 7.33 (s, 1H), 5.95-5.64 (m, 3H), 5.38-5.01 (m, 6H), 4.52-3.84 (m, 12H), 1.44 and 1.42 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 169.62-169.02 (5 lines), 166.75, 155.96-155.64 (2 lines), 134.16-131.07 (8 lines), 126.10-125.42 (3 lines), 119.52-116.73 (6 lines), 80.30, 51.34-49.89 (4 lines), 48.20-46.58 (7 lines); IR (KBr) 3073, 3007, 2978, 2932, 1792, 1698, 1659, 1460, 1408, 1348, 1310, 1249, 1222, 1176, 1122, 1080 cm^{-1}; HRMS (ESI-FTICR) for $[M+Na]^+$: calcd 610.1249; found 610.1290. Anal. calcd for $C_{26}H_{32}N_3O_6Cl_3$: C, 53.03; H, 5.48; N, 7.14. Found: C, 53.42, H, 5.49; N, 7.06%.

t-Boc-(Nall)Gly-(Nall)Gly-(Nall)Gly-(Nall)Gly-OEt **3-21**

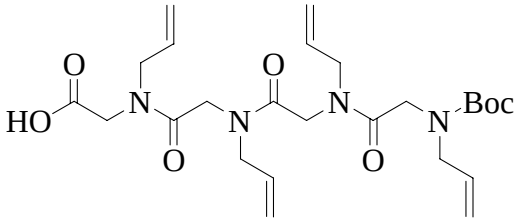


Following general EDCI coupling procedures, acid **3-18** (8.60 g, 21.0 mmol), ester **3-13** (2.73 g, 19.1 mmol), EDCI (5.15 g, 26.9 mmol), HOBt (3.63 g, 26.9 mmol), DMAP (235 mg, 1.92 mmol), DIPEA (10 mL, 57 mmol), and CH_2Cl_2 (160 mL) gave a viscous

slightly yellow oil. Purification by column chromatography on silica gel with EtOAc/Hexane (5:5 to 7:3) yielded ester **3-21** (9.06 g, 87%) as a colorless oil.

3-21: R_f = 0.27 (EtOAc/hexane, 7:3); ^{1}H NMR ($CDCl_3$) δ 5.83-5.58 (m, 4H), 5.25-4.97 (m, 8H), 4.22-3.80 (m, 18H), 1.37 and 1.35 (s, 9 H), 1.24-1.16 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 169.04, 168.52, 168.15, 155.65, 133.92-132.11 (5 lines), 118.58-116.38 (7 lines), 79.93, 61.75-61.21 (2 lines), 50.82-48.24 (7 lines), 47.60-46.50 (4 lines), 28.33, 14.17; IR (KBr) 3083, 2981, 1747, 1669, 1467, 1366, 1197, 1026 cm^{-1}; HRMS for $C_{27}H_{43}N_4O_7$ $[M]^+$: calcd 535.3132, found 535.3115.

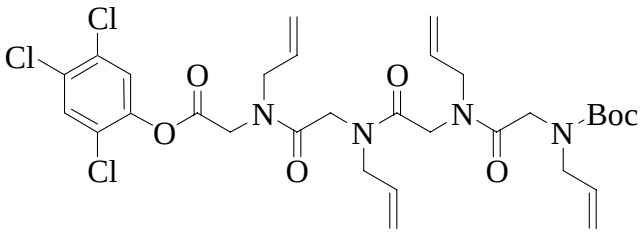
t-Boc-(Nall)Gly-(Nall)Gly-(Nall)Gly-(Nall)Gly-OH **3-22**



Following general hydrolysis procedures, ester **3-21** (2.48 g, 4.64 mmol), 4 *N* NaOH (1.4 mL), MeOH (5.6 mL), and 1 *N* HCl (5.6 mL) yielded **3-22** (2.0 g, 85%) as a white foam. Acid **3-22** was used in the next step without further purification.

3-22: ^{1}H NMR ($CDCl_3$) δ 11.00 (s, 1H), 5.82-5.58 (m, 4H), 5.23-4.97 (m, 8H), 4.26-3.70 (m, 16H), 1.36 and 1.35 (s, 9H); ^{13}C ($CDCl_3$) δ 170.75, 169.63, 168.95, 168.68, 155.80, 155.43, 133.66-131.80 (6 lines), 118.50, 117.92, 117.23, 116.64, 80.30, 50.65, 50.34, 49.87, 47.12, 46.76, 28.29.

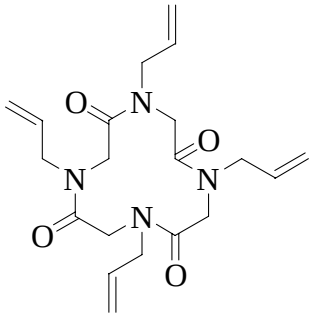
Boc protected 2,4,5-trichlorophenol ester **3-23**



Following general EDCI coupling procedures, acid **3-22** (1.80 g, 3.55mmol), 2,4,5-trichlorophenol (**3-19**) (0.636 g, mmol), EDCI (0.845 g, 4.41 mmol), HOBt (0.577 g, 4.27 mmol), DMAP (0.060 g, 0.49 mmol), DIPEA (1.7 mL, 9.7 mmol), and CH_2Cl_2 (30 mL) gave a viscous slightly yellow oil. Purification by column chromatography on silica gel with Et_2O/hexane (8:2 to 10:0) yielded **3-22**(1.2 g, 51%) as a white semi-solid.

3-22: R_f = 0.21 (Et_2O); ^{1}H NMR ($CDCl_3$) δ 7.48-7.23 (m, 2H), 5.85-5.55 (m, 4H), 5.25-4.4.95 (m, 8H), 4.35-3.80 (m 16H), 1.38 (s, 9H); ^{13}C NMR (CDCl3) δ 169.34, 168.97, 168.78, 166.54, 155.80, 145.46, 133.98-130.43 (9 lines), 125.97, 125.35, 119.48-116.52 (6 lines), 80.15, 51.21-50.05 (4 lines), 47.67-46.71 (3 lines), 28.45; IR (neat) 3085, 2979, 2931, 1784, 1673, 1460 1405, 1366, 1350, 1222, 1171, 1122, 1082 cm^{-1}; HRMS (ESI-FTICR) for $[M+Na]^+$: calcd 707.1778, found 707.1762.

Tetramer Scaffold **3-5**

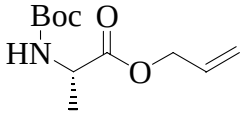


A solution of trifluoroacetic acid (4 mL) and CH_2Cl_2 (10 mL) was added drop-wise to a stirred solution of ester **3-23** (816 mg, 1.19 mmol) and CH_2Cl_2 (10 mL). After stirring for 45 min, the solvent and excess TFA were removed *in vacuo*, followed by neutralization with $NaHCO_3$, and extraction with EtOAc. The organic layer was washed with brine and dried (Na_2SO_4) to give a viscous oil. The oil was resuspended in anhydrous dioxane (300 mL) and pyridine (0.1 mL, 1.2 mmol), and the solution was heated at 100 °C for 24 h. Concentration *in vacuo* and purification by column

chromatography with EtOAc/Hexane (5:5 to 9:1) gave tetramer **3-5** (120 mg, 26%) as a white solid.

3-5: R_f = 0.23 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 5.81-5.67 (m, 4H), 5.31-5.22 (m, 8H), 4.04-3.96 (m, 16H); ^{13}C NMR ($CDCl_3$) δ 163.38, 130.94, 119.88, 49.34, 48.41; IR (KBr) 3294, 3081, 2932, 1658, 1487, 1415, 1338, 1297, 1195, 1162, 1128, 1074, 1011 cm^{-1}; HRMS (CI pos) for $C_{20}H_{29}N_4O_4$ $[M+H]^+$: calcd 389.2188; found 389.2198. Anal. calcd for $C_{20}H_{28}N_4O_4$: C, 61.84; H, 7.27; N, 14.42. Found C, 61.74; H, 7.40; N, 14.00%.

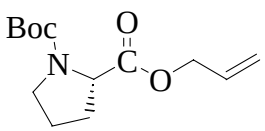
t-Boc-allyl ester of alanine **3-26b**



Following amino acid coupling procedures, t-Boc-L-alanine **3-24b** (1.73 g, 9.14 mmol), DIC (2.8 mL, 18 mmol), DMAP (0.230 g, 1.89 mmol), HOBt (1.27 g, 9.40 mmol), allyl alcohol (1.0 mL, 15 mmol), and CH_2Cl_2 (15 mL) yielded **3-26b** (2.0 g, 95%) as a colorless oil.

3-26b: R_f = 0.42 (hexane/EtOAc, 8:2); $[\alpha]^{25}_D$ = -35.0° (c = 1.04, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.89 (ddt, J = 17.0, 10.2, 5.6 Hz, 1H), 5.37-5.19 (m, 2H), 5.11 (br s, 1H), 4.69-4.54 (m, 2H), 4.39-4.26 (m, 1H), 1.43 (s, 9H), 1.38 (d, J = 7.1, 3H); ^{13}C NMR ($CDCl_3$) δ 173.19, 155.23, 131.78, 118.72, 79.95, 65.94, 49.39, 28.47, 18.81; IR (neat) 3368, 2980, 2937, 1716, 1650, 1518, 1455, 1367, 1251, 1167, 1069; HRMS (ESI-FTICR) for $[M+Na]^+$, calcd 252.1206, found 252.1227. Anal. calcd for $C_{11}H_{19}NO_4$: C, 57.62; H, 8.35; N, 6.11. Found: C, 57.88; H, 8.77; N, 6.47.

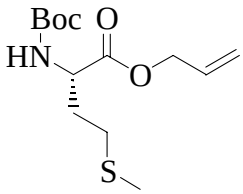
t-Boc-allyl ester proline **3-26c**



Following amino acid coupling procedures, t-Boc-L-proline **3-24c** (4.15 g, 19.3 mmol), DIC (6.0 mL, 39 mmol), DMAP (0.707 g, 5.79 mmol), HOBt (2.74 g, 20.3 mmol), allyl alcohol (2.24 g, 38.6 mmol), and CH_2Cl_2 (32 mL) yield **3-26c** (4.7 g, 95%) as a clear oil.

3-26c: R_f = 0.25 (hexane/EtOAc, 4:1); $[\alpha]^{25}_D$ = -70.9° (c = 1.00, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.89 (ddt, J = 16.7, 10.5, 5.7 Hz, 1H), 5.37-5.15 (m, 2H), 4.68-4.51 (m, 2H), 4.36-4.18 (m, 1H), 3.58-3.30 (m, 2H), 2.28-2.09 (m, 1H) 2.02-1.71 (m, 3H), 1.43 and 1.38 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.99, 153.89, 131.96, 118.73-118.24 (2 lines), 80.00-79.86 (2 lines), 65.58, 59.28-58.98 (s lines), 46.68-46.46 (2 lines) 31.05-30.08 (2 lines), 28.57-28.45 (2 lines), 24.46-23.77 (2 lines); IR (KBr) 2978, 2882, 1749, 1702, 1397, 1258, 1162, 1122, 1089; HRMS (CI pos) for $C_{13}H_{21}NO_4$ $[M+H]^+$, calcd 256.1549, found 256.1541.

t-Boc-allyl ester of methionine **3-26d**

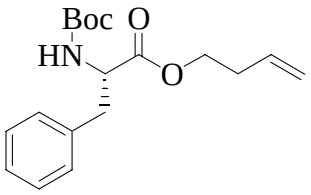


Following amino acid coupling procedures, t-Boc-L-methionine **3-24d** (2.23 g, 8.94 mmol), DIC (2.8 mL, 18 mmol), DMAP (0.300 g, 2.46 mmol), HOBt (1.26 g, 9.33 mmol), allyl alcohol (0.90 mL, 13 mmol), and CH_2Cl_2 (15 mL) yielded **3-26d** (2.5 g, 97%) as a colorless oil.

3-26d: R_f = 0.28 (hexane/EtOAc, 8:2); $[\alpha]^{25}_D$ = -32.4° (c = 1.04, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.88 (ddt, J = 17.1, 10.4, 5.7 Hz, 1H), 5.35-5.16 (m, 3H), 4.67-4.55 (m, 2H), 4.44-4.34 (m, 1H), 2.51 (t, J = 7.5 Hz, 2H), 2.18-1.83 (m, 5H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.11, 155.44, 131.67, 119.01, 80.07, 66.09, 52.95, 32.26, 30.08, 28.41,

15.56; IR (neat) 3362, 2977, 2920, 1716, 1650, 1511, 1447, 1367, 1251, 1167, 1050; HRMS (CI pos) for $C_{13}H_{24}NO_4S$ $[M+H]^+$, calcd 290.1426, found 290.1421.

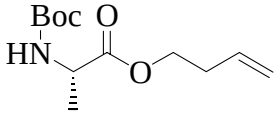
Phenylalanine derivative **3-26e**



Following amino acid coupling procedures, t-Boc-L-phenylalanine **3-24e** (5.35 g, 20.2 mmol), DIC (5.2 mL, 33.6 mmol), DMAP (0.48 g, 3.93 mmol), HOBt (2.96 g, 21.9), 3-buten-1-ol (2.7 mL, 31.4 mmol), and CH_2Cl_2 (40 mL) yielded **3-26e** (5.8 g, 90%) as a white precipitate, m.p. = 79-80.5 °C.

3-26e: R_f = 0.40 (hexane/EtOAc, 4:1); $[\alpha]^{25}_D$ = -9.01° (c = 1.00, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.33-7.10 (m, 5H), 5.72 (ddt, J = 17.1, 10.6, 6.6 Hz, 1H), 5.14-5.03 (m, 2H), 4.99 (d, J = 8.2 Hz, 1H), 4.57 (dt, J = 8.2, 6.4 Hz, 1H), 4.14 (t, J = 6.8 Hz, 2H), 3.11 (dd, J = 13.6, 6.5 Hz, 1H), 3.03 (dd, J = 13.7, 6.6 Hz, 1H), 2.40-2.29 (m, 2H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 172.06, 155.24, 136.21, 133.78, 129.50, 128.68, 127.15, 117.68, 80.01, 64.50, 54.58, 38.54, 33.01, 28.45; IR (KBr) 3355, 3077, 3030, 3006, 2973, 2930, 1735, 1708, 1645, 1516, 1455, 1391, 1365, 1288, 1220, 1187, 1086, 1054, 1020; Anal. calcd for $C_{18}H_{25}NO_4$: C, 67.69; H, 7.89; N, 4.39. Found: C, 67.83; H, 8.07; N, 4.36%.

Alanine derivative **3-26f**

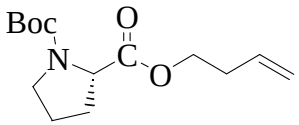


Following amino acid coupling procedures, t-Boc-L-alanine **3-24f** (1.86 g, 9.83 mmol), DIC (2.5 mL, 16 mmol), DMAP (0.240 g, 1.96 mmol), HOBt (1.45 g, 10.7

mmol), 3-buten-1-ol (1.3 mL, 16 mmol), and CH_2Cl_2 (20 mL) yielded **3-26f** (2.4 g, 98%) as a colorless oil.

3-26f: R_f = 0.35 (hexane/EtOAc, 4:1); $[\alpha]^{25}_D$ = -45.7° (c = 1.11, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.75 (ddt, J = 17.1, 10.5, 6.8, 1H), 5.16-5.00 (m, 3H), 4.34-4.08 (m, 3H), 2.43-2.34 (m, 2H), 1.42 (s, 9H), 1.35 (d, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.46, 155.21, 133.76, 117.61, 79.88, 64.32, 49.35, 33.15, 28.47, 18.87; IR (KBr) 3368, 2980, 1718, 1644, 1517, 1168, 1069; HRMS (CI pos) for $C_{12}H_{22}NO_4$ $[M+H]^+$: calcd 244.1549 , found 244.1549.

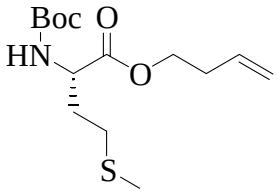
Proline derivative **3-26g**



Following amino acid coupling procedures, t-Boc-L-proline **3-24g** (4.06 g, 18.9 mmol), DIC (5.0 mL, 32 mmol), DMAP (0.730 g, 5.98 mmol), HOBt (2.82 g, 20.9 mmol), 3-buten-1-ol (2.7 mL, 32 mmol), and CH_2Cl_2 (31 mL) yield **3-26g** (4.5 g, 89%) as a clear oil.

3-26g: R_f = 0.41 (hexane/EtOAc, 7:3); $[\alpha]^{25}_D$ = -72.3° (c = 1.24, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.71 (ddt, J = 17.0, 10.2, 6.8 Hz, 1H), 5.11-4.96 (m, 2H), 4.27-4.01 (m, 3H), 3.54-3.26 (m, 2H), 2.38-2.27 (m, 2H), 2.21-1.73 (m, 4H), 1.39 and 1.34 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 173.21-172.95 (2 lines), 153.87, 134.05-133.83 (2 lines), 117.46-117.23 (2 lines), 79.89-79.76 (2 lines), 63.93, 59.28-58.98 (2 lines), 46.63-46.41 (2 lines), 33.20, 31.03-30.08 (2 lines), 28.54-28.45 (2 lines), 24.37-23.67 (2 lines); IR (KBr) 3482, 3080, 2977, 1699, 1395, 1160; HRMS (CI pos) for $C_{14}H_{23}NO_4$ $[M+H]^+$: calcd 270.1705, found 270.1701.

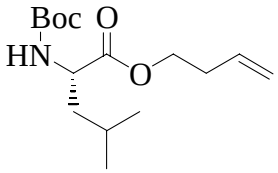
Methionine derivative **3-26h**



Following amino acid coupling procedures, t-Boc-L-methionine **3-24h** (5.59 g, 22.4 mmol), DIC (5.2 mL, 34 mmol), DMAP (0.577 g, 4.72 mmol), HOBt (3.35 g, 24.8 mmol), 3-buten-1-ol (2.9 mL, 34 mmol), and CH_2Cl_2 (40 mL) yield **3-26h** (6.0 g, 88 %) as a clear oil.

3-26h: R_f = 0.26 (hexane/EtOAc, 8:2); $[\alpha]^{25}_D$ = -23.8° (c = 1.10, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.76 (ddt, J = 17.5, 10.3, 6.7 Hz, 1H), 5.16-5.05 (m, 3H), 4.44-4.33 (m, 1H), 4.26-4.13 (m, 2H,), 2.52 (t, J = 7.6 Hz, 2H), 2.40 (qt, J = 6.7, 1.2, 2H), 2.18-1.86 (m, 5H); 1.44 (s, 9H); ^{13}C NMR ($CDCl_3$) 172.40, 155.46, 133.75, 117.74, 80.13, 64.59, 53.02, 33.16, 32.49, 30.15, 28.50, 15.64; IR (KBr) 3362, 3079, 2978, 2919, 1716, 1643, 1509, 1446, 1391, 1367, 1251; HRMS (CI pos) for $C_{14}H_{26}NO_4S$ $[M+H]^+$, calcd 304.1582 , found 304.1570.

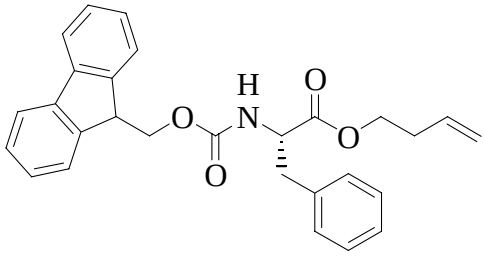
Leucine derivative **3-26i**



Following amino acid coupling procedures, t-Boc-L-leucine **4-24i** (2.08 g, 8.99 mmol), DIC (2.0 mL, 13 mmol), DMAP (0.179 g, 1.47 mmol), HOBt (1.44 g, 10.6 mmol), 3-buten-1-ol (1.0 mL, 12 mmol), and CH_2Cl_2 (50 mL) yield **4-26i** (2.1 g, 83%) as a clear oil.

3-26i: R_f = 0.33 (hexane/EtOAc, 9:1); $[\alpha]^{25}_D$ = -39.2° (c = 1.42, MeOH); ^{1}H NMR ($CDCl_3$) δ 5.72 (ddt, J = 17.3, 10.1, 6.7 Hz, 1H), 5.10-4.92 (m, 3H), 4.28-4.05 (m, 3H), 2.35 (qt, J = 6.7, 1.1 Hz, 2H), 1.71-1.39 (m, 12H), 0.90 (d, J = 1.2, 3H), 0.88 (d, J = 1.3 Hz, 3 H); ^{13}C NMR ($CDCl_3$) 173.50, 155.48, 133.79, 117.48, 79.72, 64.17, 52.24, 41.97, 33.10, 28.40, 24.86, 22.87, 22.03; IR (neat) 3368, 3081, 2960, 2872, 1718, 1644, 1509, 1455, 1367, 1165, 1122, 1048, 1023; Anal. calcd for $C_{15}H_{27}NO_4$: C, 67.69; H, 7.89; N, 4.39. Found: C, 67.83; H, 8.07; N, 4.36%.

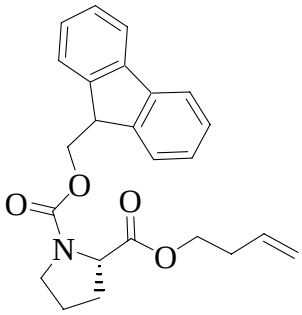
Fmoc-L- Phenylalanine Derivative **3-27a**



Following amino acid coupling procedures, Fmoc-L-phenylalanine **3-25a** (1.00 g, 2.58 mmol), DIC (0.60 mL, 3.87 mmol), DMAP (60.0 mg, 0.491 mmol), HOBt (422 mg, 3.12 mmol), 3-buten-1-ol (0.33 mL, 3.86 mmol), and THF (5.0 mL) yield **3-27a** (1.1 g, 99 %) as a white solid, m.p. = 52-54 °C .

3-27a: R_f = 0.44 (hexane/EtOAc, 7:3); $[\alpha]^{25}_D$ = -20.0° (c = 1.06, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.85-7.16 (m, 13 H), 5.86-5.72 (m, 1H), 5.46-5.09 (m, 3H), 4.80-4.20 (m, 6H), 3.25-3.15 (m, 2H), 2.48-2.32 (m, 2H); ^{13}C NMR ($CDCl_3$) 171.61, 155.67, 143.98-143.88 (2 lines), 141.42, 135.93, 133.67, 129.47, 128.69, 127.83, 127.23-127.17 (2 lines), 125.25-125.18 (2 lines), 120.11, 117.70, 67.05, 64.60, 54.94, 47.26, 38.38, 32.94; IR (KBr) 3327, 3064, 2962, 1696, 1605, 1536, 1450, 1388, 1263, 1104, 1086, 1045; HRMS (CI pos) for $C_{28}H_{28}NO_4$ $[M+H]^+$: calcd 442.2018, found 442.2025; Anal. calcd for $C_{28}H_{27}NO_4$: C, 76.17; H, 6.16; N, 3.17. Found: C, 75.81; H, 6.22; N, 3.16%.

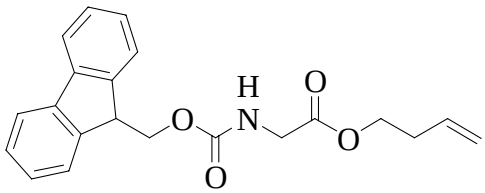
Fmoc L-Proline Derivative **3-27b**



Following amino acid coupling procedures, Fmoc-L-proline **3-25b** (1.04 g, 3.07mmol), DIC (0.71 mL, 4.6 mmol), DMAP (0.0749 g, 0.613 mmol), HOBt (0.502 g, 3.71 mmol), 3-buten-1-ol (0.40 mL, 4.7 mmol), and THF (7.0 mL) yield **3-27b** (1.1 g, 92%) as a colorless oil.

3-23b: R_f = 0.35 (hexane/EtOAc, 7:3); $[\alpha]^{25}_D$ = -49.4° (c = 1.25, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.62-7.12 (m, 8H), 5.69-5.50 (m, 1H), 5.00-4.84 (m, 2H) 4.33-3.89 (m, 6H), 3.55-3.29 (m, 2H), 2.27-1.66 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 172.43-172.36 (2 lines), 154.67-154.27 (2 lines), 144.10-143.68 (4 lines), 141.18-141.13 (2 lines), 133.79-133.60 (2 lines)127.58, 126.94, 125.09-124.86 (3 lines), 119.86, 117.31-117.17 (2 lines), 67.31, 63.85, 59.20-58.75 (2 lines), 47.20-46.34 (4 lines), 32.96-32.91 (2 lines) 30.96-29.80 (2 lines) 24.19, 23.17; IR (KBr) 3068, 2957, 2884, 1745, 1705, 1451, 1417, 1349, 1194, 1120, 1089; HRMS (ESI-FTICR) for $[M+Na]^+$: calcd 414.1676, found 414.1669; Anal. calcd for $C_{24}H_{25}NO_4$: C, 73.64; H, 6.44; N, 3.58. Found: C, 73.28; H, 6.61; N, 3.54%.

Fmoc Glycine Derivative **3-27c**



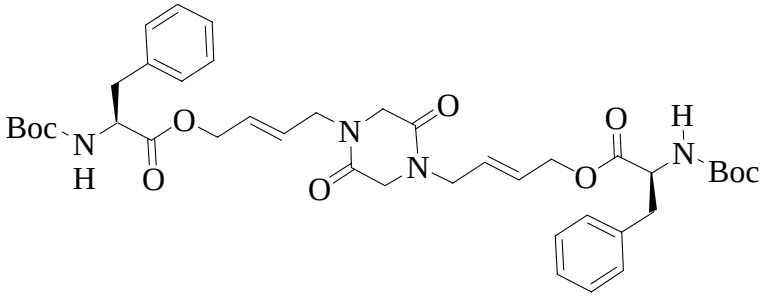
Following amino acid coupling procedures, Fmoc-glycine **3-25c** (1.90 g, 6.39 mmol), DIC (1.5 mL, 9.7 mmol), DMAP (0.318 g, 2.60 mmol), HOBt (1.38 g, 10.2

mmol), 3-buten-1-ol (0.85 mL, 9.9 mmol), and THF (15 mL) yield **4-27c** (2.2 g, 85 %) as a white solid, m.p. = 78.5-80 °C.

3-27c: R_f = 0.40 (hexane/EtOAc, 7:3); ^{1}H NMR ($CDCl_3$) δ 7.81-7.30 (m, 8 H), 5.79 (ddt, J = 17.2, 10.2, 6.7 Hz, 1H), 5.49-5.41 (m, 1H), 5.18-5.08 (m, 2H), 4.43 (d, J = 7.0 Hz), 4.28-4.20 (m, 3H), 4.00 (d, J = 5.6 Hz, 2H), 2.42 (qt, J = 6.8, 1.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) 170.15, 156.43, 143.93, 141.40, 133.63, 127.83, 127.19, 125.20, 120.10, 117.71, 67.28, 64.54, 47.21, 42.85, 33.02; IR (KBr) 3335, 3065, 3017, 2947, 1767, 1685, 1541, 1451, 1414, 1389, 1361, 1288, 1192, 1104, 1081, 1055; HRMS (CI pos) for $C_{21}H_{22}NO_4$ $[M+H]^+$: calcd 352.1549, found 352.1556; Anal. calcd for $C_{21}H_{21}NO_4$: C, 71.78; H, 6.02; N, 3.99. Found: C, 71.62; H, 6.06; N, 3.98%.

6.2.6 General procedure for cross-metathesis of dimer **3-3** with an amino acid derivative

Cross-metathesis product t-Boc-allylester phenylalanine-dimer **3-28a**

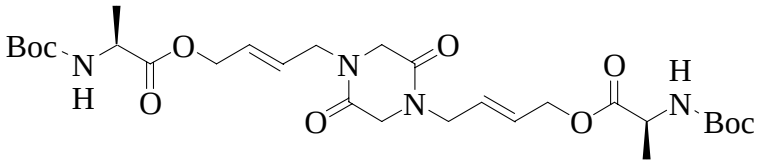


A solution of catalyst **1-4** (46 mg, 0.054 mmol) and $CHCl_3$ (0.50 mL) was added to a stirred solution of dimer **3-3** (105 mg, 0.541 mmol), amino acid **3-26a** (825 mg, 2.70 mmol), and $CHCl_3$ (0.50 mL). The reaction was heated at reflux for 10 h while flushing the headspace with argon to remove evolved ethylene. The reaction was allowed to cool to room temperature and quenched with EVE (*ca*. 0.5 mL). The solution was stirred for 30 min and concentrated under reduced pressure. Purification by column

chromatography on silica gel with hexane/EtOAc (9:1-4:6) yielded **3-28a** as an oil (150 mg, 37%) and homodimer **3-30a** (301 mg, 38%) as a white solid.

3-28a: R_f = 0.33 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.35-7.09 (m, 10H), 5.78-5.57 (m, 4H), 5.03 (d, J = 7.9 Hz, 2H), 4.66-4.51 (m, 6H), 4.07-3.90 (m, 8H), 3.16-2.98 (m, 4H), 1.41 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 171.70, 163.08, 155.14, 135.99, 129.39, 128.80, 128.65, 127.40, 127.13, 80.07, 64.56, 54.57, 49.34, 47.03, 38.41, 28.40; IR (KBr) 3324, 2978, 1666, 1498, 1366, 1169, 1022 cm^{-1}; HRMS (ESI-FTICR) for $[M+Na]^+$: calcd 771.3576, found 771.3544.

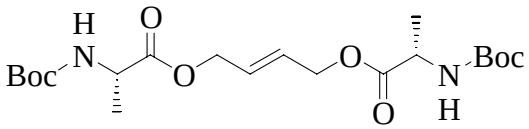
Cross-metathesis product t-Boc-allylester alanine-dimer **3-28b**



Following general CM procedures, dimer **3-3** (104 mg, 0.533 mmol), amino acid **4-26b** (600 mg, 2.62 mmol), Grubbs' catalyst **1-4** (46 mg, 0.054 mmol), and $CHCl_3$ (0.5 mL) gave a brown residue. Purification by column chromatography on silica gel with hexane/EtOAc (9:1 to 6:4, 2:8 to 0:10) yielded **3-28b** (130 mg, 40%) as an oil and homodimer **3-30b** (180 mg, 42%) as a white solid, m.p. = 96-97 °C.

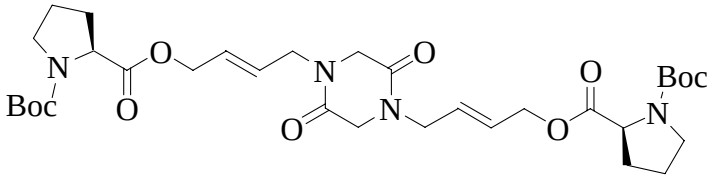
3-28b: R_f = 0.21 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 5.85-5.60 (m, 4H), 5.10 (d, J =7.2 Hz, 2H), 4.65-4.55 (m, 4H), 4.35-3.89 (m, 10H), 1.40 (s, 18H), 1.35 (d, J = 7.3 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 173.13, 163.14, 155.17, 128.93, 127.24, 79.95, 64.49, 51.52, 49.30, 47.03, 28.41, 18.61; IR (KBr) 3330, 2980, 2935, 2361, 2250, 1666, 1520, 1478, 1366, 1252, 1165, 1070, 1023 cm^{-1}; HRMS (EI pos) for $C_{28}H_{44}N_4O_4$ $[M]^+$: calcd 619.2950, found 619.2946.

Homodimer t-Boc-allyl ester alanine **3-30b**



3-30b: R_f = 0.34 (hexane/EtOAc, 6:4); ^{1}H NMR ($CDCl_3$) δ 5.87-5.78 (m, 2H) 5.11 (d, J = 7.2 Hz, 2H), 4.64-4.57 (m, 4H), 4.35-4.22 (m, 2H), 1.40 (s, 18H), 1.36 (d, J = 7.2 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 173.12, 155.19, 127.91, 79.95, 64.60, 49.32, 28.43, 18.67; IR (KBr) 3370, 2983, 2938, 1737, 1685, 1522, 1456, 1369, 1274, 1163, 1085, 1024 cm^{-1}; HRMS (CI pos) for $C_{20}H_{35}N_2O_8$ $[M+H]^+$: calcd 431.2393, found 431.2379.

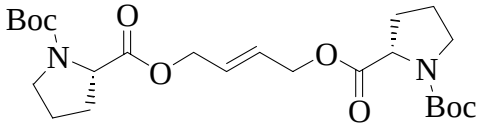
Cross-metathesis product t-Boc-allylester proline-dimer **3-28c**



Following general CM procedures, dimer **3-3** (95.5 mg, 0.492 mmol), amino acid **3-26c** (726 mg, 2.51 mmol), Grubbs' catalyst **1-4** (42.0 mg, 0.0495 mmol), and $CHCl_3$ (1.0 mL) gave a brown residue. Purification by column chromatography on silica gel with Hexane/EtOAc (9:1 to 7:3, 2:8 to 0:10) yielded **3-28c** (135 mg, 42% NMR yield) as an oil and homodimer **3-30c** (188 mg, 31%) as an oil.

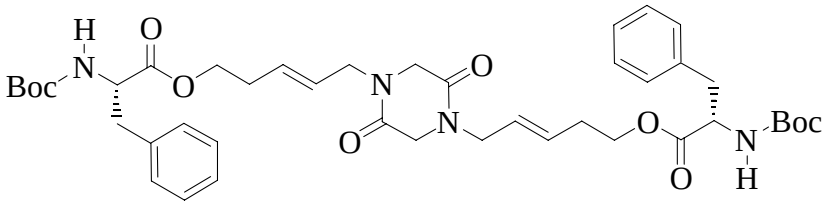
3-26c: R_f = 0.28 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 5.88-5.65 (m, 4H), 4.66-4.58 (m, 4H), 4.36-4.20 (m, 2H), 4.07-3.92 (m, 8H), 3.60-3.35 (m, 4H), 2.30-2.13 (m, 2H), 2.02-1.82 (m, 6H), 1.46 and 1.41 (s, 18H).

Homodimer t-Boc-allyl ester proline **3-30c**



3-30c: R_f = 0.37 (hexane/EtOAc, 5:5); ^{1}H NMR ($CDCl_3$) δ 5.94-5.60 (m, 2H), 4.76-4.52 (m, 4H), 4.36-4.18 (m, 2H), 3.58-3.32 (m, 4H), 2.29-2.10 (m, 2H), 2.02-1.77 (m, 6H), 1.44 and 1.38 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 172.92, 172.67, 154.48, 153.83, 128.46-127.61 (3 lines), 80.00, 64.41, 64.27, 59.22, 58.92, 46.68, 46.46, 31.04, 30.06, 28.55, 28.45, 24.47, 23.78 cm^{-1}; IR (neat) 3482, 2974, 1950, 1747, 1698, 1399, 1259, 1170 cm^{-1}. HRMS (CI pos) for $C_{24}H_{39}N_2O_8$ $[M+H]^+$: calcd 483.2706, found 483.2724. Anal. calcd for $C_{24}H_{38}N_2O_8$: C, 59.73; H, 7.94; N, 5.81. Found: C, 60.05; H, 8.20; N, 5.70%

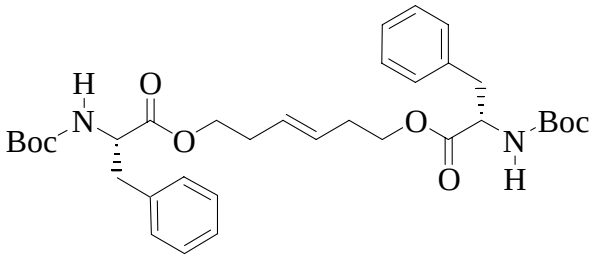
Cross-metathesis product t-Boc-homoallylester phenylalanine-dimer **3-28e**



Following general CM procedures, dimer **3-3** (103 mg, 0.530 mmol), amino acid **3-26e** (908 mg, 2.84 mmol), catalyst **1-4** (45.9 mg, 0.0541 mmol) and $CHCl_3$ (1.0 mL) gave a brown residue. Purification by chromatography on silica gel with hexane/EtOAc (100:0 – 0:100) gave **3-28e** (180 mg, 44%) as a silver foam and homodimer **3-30e** (440 mg, 50%) as a gray solid, m.p. = 142-144 °C.

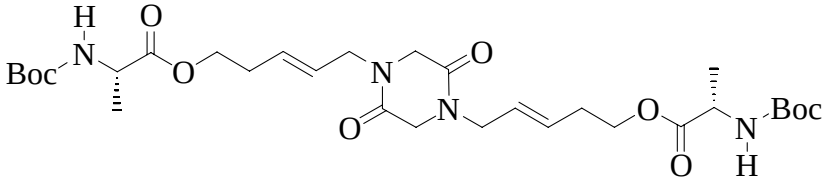
3-28e: R_f = 0.27 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.38-7.08 (m, 10H), 5.58 (dt, J = 15.2, 6.3 Hz, 2H), 5.42 (dt, J = 15.3, 6.3 Hz, 2H), 5.10 (d, J = 8.0 Hz, 2H), 4.63-4.47 (m, 2H), 4.18-3.83 (m, 12H), 3.15-2.96 (m, 4H), 2.39-2.25 (m, 4H), 1.41 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 172.01, 163.28, 155.21, 136.22, 131.46, 129.39, 128.63, 127.09, 125.68, 79.92, 64.11, 54.62, 49.11, 47.40, 38.41, 31.56, 28.40; IR (KBr) 3328, 2976, 2249, 1713, 1664, 1498, 1366, 1170, 1052 cm^{-1}; HRMS (ESI-FTICR) for $[M+Na]^+$: calcd 799.3889; found 799.3879.

Homodimer t-Boc-homoallylester phenylalanine **3-30e**



3-30e: R_f = 0.30 (hexane:EtOAc, 7:3); ^{1}H NMR ($CDCl_3$) δ 7.35-7.10 (m, 10H); 5.47-5.35 (m, 2H), 5.09 (d, J = 7.2 Hz, 2H), 4.56 (dt, J = 7.6, 7.0 Hz, 2H) 4.08 (t, J = 6.8 Hz, 4H), 3.09 (dd, J = 13.6, 6.5 Hz, 2H), 3.02 (dd, J = 13.6, 6.2 Hz, 2H), 2.37-2.20 (m, 4H), 1.40 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 171.90, 155.13, 136.17, 129.34, 128.53, 128.24, 126.99, 79.80, 64.55, 54.52, 38.39, 31.81, 28.33; IR (KBr) 3365, 3003, 2971, 2931, 1709, 1517, 1456, 1391, 1365, 1222, 1184, 1087, 1053, 1019 cm^{-1}; HRMS (ESI-FTICR) for $[M+Na]^+$: calcd 633.3146, found 633.3156.

Cross-metathesis product t-Boc-homoallylester alanine-dimer **3-28f**

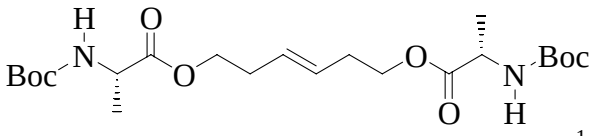


Following general CM procedures, dimer **3-3** (200 mg, 1.03 mmol), amino acid **3-26f** (1200 mg, 4.94 mmol), Grubbs' catalyst **1-4** (84.0 mg, 0.099 mmol) and $CHCl_3$ (3.0 mL) gave a brown residue. Purification by chromatography on silica gel with hexane/EtOAc (100:0 – 0:100) gave **3-28f** (250 mg, 39%) as a silver foam and homodimer **3-30f** (750 mg, 66%) as an oil.

3-28f: R_f = 0.31 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 5.65 (dt, J = 15.4, 6.8 Hz, 2H), 5.46 (dt, J = 15.3, 6.4 Hz, 2H), 5.16 (s, 2H), 4.28-4.07 (m, 6H), 4.01-3.90 (m, 8H), 2.44-2.37 (m, 4H), 1.43 (s, 18H), 1.36 (d, J = 7.7, 6H); ^{13}C NMR (CDCl3) δ 173.20, 163.22,

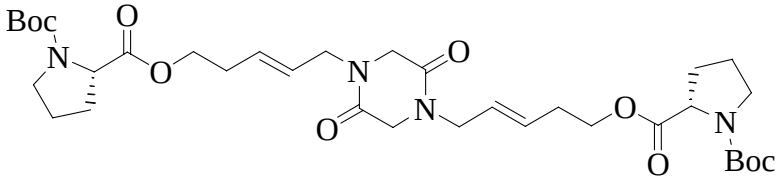
155.10, 131.37, 125.49, 79.45, 63.73, 49.13, 48.87 47.15, 31.51, 28.24, 22.81, 18.20; IR (neat) 3327, 2979, 2934, 1666, 1521, 1478, 1391, 1367, 1335, 1250, 1166, 1115, 1068, 1023 cm^{-1}; HRMS (CI pos) for $C_{30}H_{49}N_4O_{10}$ $[M+H]^+$: calcd 625.3449, found 625.3450.

Homodimer t-Boc-homoallylester alanine **3-30f**



3-30f: R_f = 0.39 (hexane/EtOAc, 7:3); ^1H NMR ($CDCl_3$) δ 5.47-5.39 (m, 2H) , 5.15 (s, 2H), 4.28-4.02 (m, 6H), 2.38-2.24 (m, 4H), 1.37 (s, 18H), 1.30 (d, J = 7.3 Hz, 6H); ^{13}C NMR (CDCl3) δ 173.41, 155.19, 131.70, 128.29, 127.39 (*cis*), 79.81, 64.47, 49.31, 32.01, 28.43, 18.73; IR 3366, 2979, 1715, 1518, 1455, 1392, 1367, 1251, 1166, 1069, 1025 cm^{-1}; HRMS (CI pos) for $C_{22}H_{39}N_2O_8$ $[M+H]^+$: calcd 459.2706, found 459.2705.

Cross-metathesis product t-Boc-homoallylester proline-dimer **3-28g**

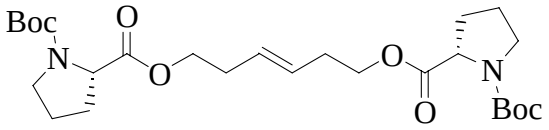


Following general CM procedures, dimer **3-3** (mg, mmol), amino acid **3-26g** (mg, mmol), Grubbs' catalyst **1** (mg, mmol) and $CHCl_3$ (mL) gave a brown residue. Purification by chromatography on silica gel with hexane/EtOAc (100:0 – 0:100) gave **3-28g** (140 mg, 45%) and homodimer **3-30g** (325 mg, 55%) as an oil.

3-28g: R_f = 0.28 (EtOAc); ^1H NMR ($CDCl_3$) δ 5.68-5.31 (m, 4H), 4.24-3.75 (m, 14H), 3.52-3.24 (m, 4H), 2.44-1.73 (m, 12H), 1.39 and 1.34 (s, 18H); ^{13}C NMR (CDCl3) δ 173.13, 172.92, 163.24, 163.14, 154.36, 153.75, 131.75, 131.41, 125.60, 125.38, 79.83, 79.69, 63.62, 59.14, 58.81, 49.05, 47.35, 46.58, 46.34, 31.66, 30.93, 29.99, 28.46, 28.36,

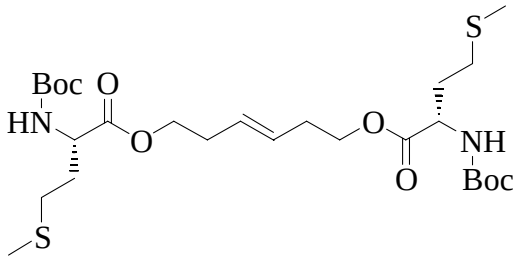
24.32, 23.64; IR (neat) 3494, 2975, 1745, 1670, 1477, 1399, 1279, 1162 cm^{-1}; HRMS (CI pos) for $C_{34}H_{53}N_4O_{10}$ $[M+H]^+$: calcd 677.3762, found 677.3751.

Homodimer t-Boc-homoallylester proline **3-30g**



3-30g: R_f = 0.33 (hexane/EtOAc, 7:3); ^1H NMR ($CDCl_3$) δ 5.50-5.32 (m, 2H), 4.26-3.92 (m, 6H), 3.54-3.20 (m, 4H), 2.36-1.74 (m, 12H), 1.39 and 1.34 (s, 18H); ^{13}C NMR (CDCl3) δ 173.10, 172.82, 154.32, 153.73, 128.43, 128.22, 127.98, 79.71, 79.61, 64.07, 59.11, 58.81, 46.51, 46.28, 31.97, 30.89, 29.94, 28.40, 28.30, 26.86, 24.25, 23.55 IR (neat) 3522, 2976, 2882, 1747, 1700, 1478, 1455, 1397, 1258, 1162, 1122 cm^{-1}; HRMS (CI pos) for $C_{26}H_{43}N_2O_8$ $[M+H]^+$: calcd 511.3019, found 511.3017.

Homodimer methionine t-Boc-homoallylester **3-30h**

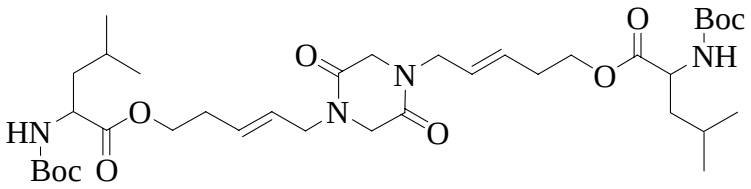


Following general CM procedures, dimer **3-3** (86.0 mg, 0.443 mmol), amino acid **3-26h** (690 mg, 2.27 mmol), Grubbs' catalyst **1-4** (39.9 mg, 0.0470 mmol) and $CHCl_3$ (1.0 mL) gave a brown residue. Purification by chromatography on silica gel with hexane/EtOAc (9:1) gave homodimer **3-30h** (94 mg, 14%) as an oil.

3-30h: R_f = 0.31 (hexane/EtOAc, 7:3); ^1H NMR ($CDCl_3$) δ 5.78-5.39 (m, 2H), 5.19 (s, 2H), 4.44-4.30 (m, 2H), 4.22-4.07 (m, 4H), 2.56-2.29 (m, 8H), 2.17-1.83 (m, 10H), 1.42 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 172.39, 155.48, 128.42-126.62 (3 lines), 80.09, 65.63-64.19 (4 lines), 52.98, 32.38-31.69 (4 lines), 30.14-29.80 (2 lines), 28.45, 27.19-26.95 (2

lines), 15.59; IR (neat) 3357, 2976, 1715, 1515, 1366, 1252, 1166, 1051 cm^{-1}; HRMS (CI pos) for $C_{26}H_{47}N_2O_8S_2$ $[M+H]^+$: calcd 579.2774, found 579.2768.

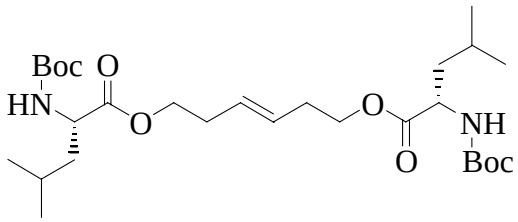
Cross-metathesis product t-Boc-homoallylester leucine-dimer **3-28i**



Following general CM procedures, dimer **3-3** (95.0 mg, 0.489 mol), amino acid **3-26i** (682 mg, 2.39 mmol), Grubbs' catalyst **1-4** (43.5 mg, 0.0512 mmol) and $CHCl_3$ (1.0 mL) gave a brown residue. Purification by chromatography on silica gel with hexane/EtOAc (9:1 – 1:9) gave **3-28i** (103 mg, 30%) and homodimer **3-30i** (381 mg, 59%) as an oil.

3-28i: R_f = 0.42 (EtOAc:hexane, 8:2); ^{1}H NMR ($CDCl_3$) δ 5.72-5.41 (m, 4H), 5.03 (d, J = 7.6 Hz, 2H), 4.31-3.90 (m, 14H), 2.53-2.35 (m, 4H), 1.86-1.38 (m, 24H), 0.94 (d, J = 6.72, 12H); ^{13}C NMR (CDCl3) δ 173.58, 163.41, 155.60, 131.74, 125.74, 79.90, 64.01, 52.35, 49.21, 47.53, 41.85, 31.84, 28.54, 25.00, 23.04, 22.09; IR 3325, 2960, 1713, 1665, 1522, 1475, 1390, 1366, 1334, 1253, 1166, 1048, 1020 cm^{-1}. HRMS (CI pos) for $C_{36}H_{60}N_4O_{10}$ $[M+H]^+$: calcd 709.4388, found 709.4390.

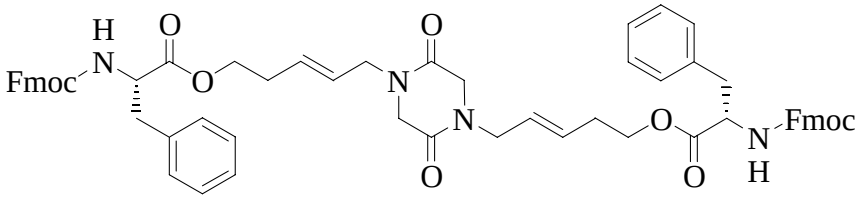
Homodimer t-Boc-homoallylester leucine **3-30i**



3-30i: R_f =0.29 (hexane/EtOAc, 8:2); 1H NMR ($CDCl_3$) δ 5.53-5.36 (m, 2H), 4.97 (d, J = 6.4 Hz, 2H), 4.30-4.00 (m, 6H), 2.41-2.26 (m, 4H), 1.73-1.37 (m, 24H), 0.90 (d, J = 6.3 Hz, 12H); ^{13}C NMR ($CDCl_3$) δ 173.49, 155.49, 128.33, 127.42 (*cis*) 79.77, 64.38,

52.25, 41.95, 32.05, 28.45, 26.95-24.89 (2 lines), 22.94-22.05 (2 lines); IR 3379, 2960, 1716, 1510, 1391, 1367, 1253, 1165, 1048 cm^{-1}. HRMS (CI pos) for $C_{28}H_{50}N_2O_8$ $[M+H]^+$ calcd 543.3645, found 543.3630; Anal. calcd for $C_{28}H_{50}N_2O_8$: C, 61.97; H, 9.29; N, 5.16. Found: C, 62.13; H, 9.62; N, 5.04 %

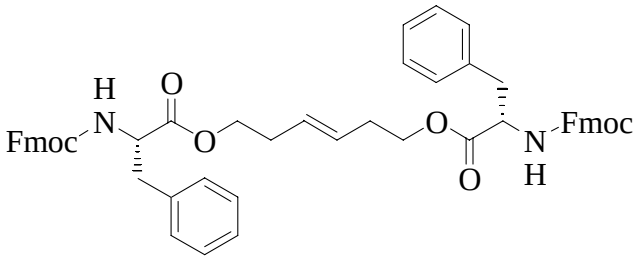
Cross-metathesis product Fmoc-homoallyl phenylalanine-dimer **3-29a**



Following general CM procedures, dimer **3-3** (61.0 mg, 0.314mol), amino acid **3-27a** (614 mg, 1.39 mmol), Grubbs' catalyst **1-4** (30.0 mg, 0.035 mmol) and $CHCl_3$ (1.5 mL) gave a brown residue. Purification by chromatography on silica gel with hexane/EtOAc (9:1 – 2:8) gave **3-29a** (136 mg, 42%) as brown foam, and homodimer **3-31a** (285 mg, 48%) as a white solid, m.p. = 54-56 °C.

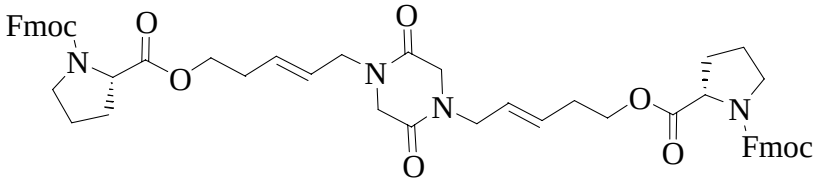
3-29a: R_f = 0.35 (EtOAc/hexane, 8:2); ^{1}H NMR ($CDCl_3$) δ 7.70-7.01 (m, 26H), 5.53-5.26 (m, 6H), 4.59-3.74 (m, 18H), 3.09-2.92 (m, 4H), 2.34-2.17 (m, 4H) ; ^{13}C NMR (CDCl3) δ 171.65, 163.35, 155.75, 143.87, 141.39, 136.02, 131.46, 129.42, 128.70, 127.82, 127.17, 125.73, 125.19, 120.09, 67.01, 64.28, 55.06, 49.10, 48.32, 47.30, 38.31, 31.63; IR 3304, 2956, 1721, 1662, 1478, 1451, 1334, 1260 cm^{-1}. HRMS (ESI-FTICR) for $[M+Na]^+$: calcd 1043.4202, found 1043.4214.

Homodimer Fmoc-homoallyl phenylalanine **3-31a**



3-31a: R_f = 0.30 (Hexane/EtOAc, 7:3); ^{1}H NMR ($CDCl_3$) δ 7.72-6.98 (m, 26H), 5.55-5.20 (m, 4H), 4.64-3.92 (m, 12H), 3.10-2.75 (m, 4H), 2.29-2.12 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 171.64, 155.73, 144.01-143.90 (2 lines), 141.46, 135.96, 129.49, 128.74, 128.38, 127.87, 127.29-127.21 (2 lines), 125.29-125.21 (2 lines), 120.15, 67.10, 64-87-64.77 (2 lines), 54.99, 47.30, 38.44, 31.93; IR 3343, 2953, 1728, 1521, 1450, 1211, 1051 cm^{-1}. HRMS (ESI-FTICR) for $[M+Na]^+$ calcd 877.3459, found 877.3485; Anal. calcd for $C_{54}H_{50}N_2O_8$: C, 75.86; H, 5.89; N, 3.28. Found: C, 75.46; H, 5.99; N, 3.26 %

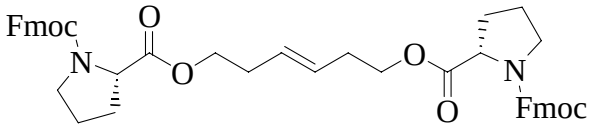
Cross-metathesis product Fmoc-homoallyl proline-dimer **3-29b**



Following general CM procedures, dimer **3-3** (63.0 mg, 0.324mol), amino acid **3-27b** (558 mg, 1.42 mmol), Grubbs' catalyst **1-4** (30.0 mg, 0.0353 mmol) and $CHCl_3$ (1.5 mL) gave a brown residue. Purification by chromatography on silica gel with hexane/EtOAc (9:1 – 2:8) gave **3-29b** (140 mg, 46% NMR yield) and homodimer **3-31b** (280 mg, 52% NMR yield) as a brown semi-solid.

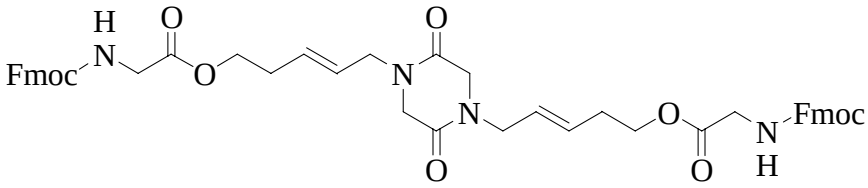
3-29b: R_f = 0.32 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.67-7.18 (m, 16H), 5.58-5.27 (m, 4H), 4.41-3.38 (m, 24H), 2.31-1.80 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 172.56, 163.27-163.15 (2 lines), 154.84-154.40 (2 lines), 144.18-143.77 (3 lines), 141.32, 131.53-131.28 (2 lines), 127.73, 127.12, 125.58-125.02 (4 lines), 120.02, 67.49, 63.84, 59.27-58.88 (2 lines), 49.06, 47.34-46.54 (4 lines), 31.64-31.59 (2 lines), 31.15, 30.00, 24.42, 23.42; HRMS (ESI-FTIR-MS) for $[M+Na]^+$: calcd 943.3899, found 943.3900 .

Homodimer Fmoc-homoallyl praline **3-31b**



3-31b: R_f = 0.37 (hexane/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 7.73-7.15 (m, 16H), 5.43-5.27 (m, 2H), 4.40-3.88 (m, 12H), 3.62-3.37 (m, 4H), 2.32-1.73 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 172.57, 154.91-154.49 (2 lines), 144.24-143.86 (2 lines), 141.37, 128.44-128.11 (3 lines), 127.77, 127.13, 125.28-125.07 (3 lines), 120.06, 67.57, 64.39, 59.38-58.97 (2 lines), 47.43-46.58 (4 lines) 32.05, 31.22, 30.06, 24.46, 23.45; HRMS (ESI-FTIR) for $[M+Na]^+$ calcd 777.3148, found 777.3129.

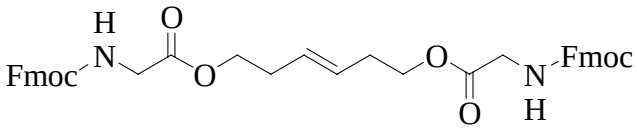
Cross-metathesis product Fmoc-homoallyl glycine-dimer **3-29c**



Following general CM procedures, dimer **3-3** (94.0 mg, 0.484 mol), amino acid **3-27c** (854 mg, 2.43 mmol), Grubbs' catalyst **1-4** (67.5 mg, 0.0795 mmol) and $CHCl_3$ (2.0 mL) gave a brown residue. Purification by chromatography on silica gel with hexane/EtOAc (9:1 – 1:9) gave **3-29c** (120 mg, 30%) as a silver solid, m.p. = 52-54 °C, and homodimer **3-31c** (357 mg, 44%) as a white solid, m.p. = 121-123 °C.

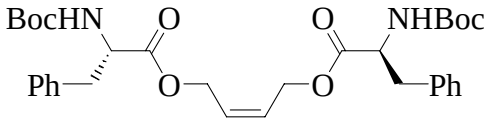
3-29c: R_f = 0.42 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.67-7.15 (m, 16H), 5.86-5.20 (m, 6H), 4.32-3.72 (m, 22H), 2.37-2.18 (m, 4H); ^{13}C NMR (CDCl3) δ 170.24, 163.54, 156.62, 143.93, 141.33, 131.84, 127.78, 127.13, 125.59, 125.23, 120.04, 67.12, 63.94, 48.89, 47.18, 42.77, 31.80; IR 3319, 3065, 2957, 1724, 1662, 1534, 1478, 1450, 1333, 1263, 1193, 1104, 1051, 1008 cm^{-1}. HRMS (ESI-FTICR) for $[M+H]^+$: calcd 841.3443, found 841.3432.

Homodimer Fmoc-homoallyl glycine **3-31c**



3-31c: R_f = 0.39 (hexane/EtOAc, 5:5); ^{1}H NMR ($CDCl_3$) δ 7.78-7.27 (m, 16H), 5.50-5.36 (m, 4H), 4.40 (d, J = 7.3 Hz, 4H), 4.26-4.14 (m, 6H), 3.98 (d, J = 5.7 Hz, 4H), 2.43-2.31 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 170.24, 156.52, 143.99, 141.48, 128.48, 127.91, 127.26, 125.27, 120.18, 67.38, 67.38, 64.80, 47.28, 42.93, 32.03; IR 3319, 2950, 1758, 1691, 1541, 1450, 1411, 1361, 1287, 1191, 1105, 1082, 1053 cm^{-1}. HRMS (ESI-FTICR) for $[M+Na]^+$ calcd 697.2520, found 697.2528; Anal. calcd for $C_{40}H_{38}N_2O_8$: C, 71.20; H, 5.68; N, 4.15. Found: C, 70.83; H, 5.74; N, 4.12%

Independent synthesis of *cis* **3-30a**

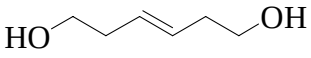


A flame dried flask under argon was charged with acid **3-24a** (2.07 g, 7.8 mmol), and CH_2Cl_2 (10 mL). The solution was cooled to 0 °C, followed by the addition of EDCI (1.47 g, 7.67 mmol), HOBt (1.05 g, 7.77 mmol), DMAP (0.095 g, 0.78 mmol), and DIPEA (1.8 mL, 10 mmol). After stirring for 20 min, (Z)-2-butene-1,4-diol (**3-33**) was added drop-wise to the solution and maintained for 6 h. The solvent was removed *in vacuo*, and the residue redissolved in EtOAc. The organic layer was washed with 1 *N* $KHSO_4$, 1 *N* $NaHCO_3$, water, and brine. The organic layer was then dried with Na_2SO_4 and concentrated *in vacuo* to give **3-30a** (1.7 g, 97%) as a white solid.

3-30a: ^{1}H NMR ($CDCl_3$) δ ^{1}H NMR ($CDCl_3$) δ 7.32-7.09 (m, 10H), 5.72-5.60 (m, 2H), 4.98 (d, J = 8.1 Hz, 2H), 4.72-4.54 (m, 6H), 3.15-2.96 (m, 4H), 1.39 (s, 18H); ^{13}C

NMR ($CDCl_3$) δ 171.81, 136.09, 129.54, 128.76, 128.09, 127.27, 80.16, 60.74, 54.64, 38.57, 28.48

Hex-3-ene, 1,6-diol **(3-35)**



Following literature procedures[60], a solution of *trans*-β-hydromuconic acid **3-34** (1.00g, 6.94 mmol), concentrated sulfuric acid (0.34 mL), and absolute methanol (50 mL) were refluxed overnight under an atmosphere of argon. The solution was cooled to room temperature and the MeOH was removed by reduced pressure. Extraction with ether, $NaHCO_3$, H_20 and brine, and drying with $MgSO_4$ gave the diester (1.0 g, 85%). A solution of the diester (1.0 g, 5.8 mmol) and THF (30 mL) was added to a reaction vessel containing $LiAlH_4$ (925 mg, 24.4 mmol) and THF (12 mL) by an addition funnel, and the reaction mixture stirred under argon at room temperature for 6 h. The reaction was quenched with EtOAc. The white precipitate that was formed was filtered off and washed with cold ether. The combined organic layers was passed through a pad of celite and concentrated under reduced pressure to give diol **3-35** (0.44 g, 65%).

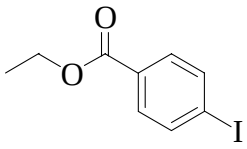
3-35: ^{1}H NMR ($CDCl_3$) δ 5.48-5.36 (m, 2H), 3.78 (s, 2H) 3.53 (t, J = 7.0 Hz, 4H); ^{13}C NMR ($CDCl_3$); 129.424, 61.648, 35.979

Independent synthesis of *trans* **3-30e**

A flame dried flask under argon was charged with acid **3-30e** (3.45 g, 13.0 mmol), and CH_2Cl_2 (40 mL). The solution was cooled to 0 °C, followed by the addition of EDCI (2.90 g, 15.1 mmol), HOBt (2.21 g, 16.4 mmol), DMAP (0.130g, 1.06 mmol), and DIPEA (4.0 mL, 23 mmol). After stirring for 20 min, diol **3-35** was added drop-wise to the solution and maintained for 16 h. The solvent was removed *in vacuo*, and the residue

redissolved in EtOAc. The organic layer was washed with 1 *N* $KHSO_4$, 1 *N* $NaHCO_3$, water, and brine. The organic layer was then dried with Na_2SO_4 and concentrated *in vacuo* to give **3-30e** (1.2 g, 54%) as a white solid. Analytical data are identical to data from CM product **3-30e**.

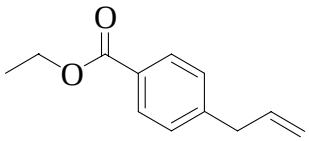
4-iodo benzoate (**4-10**)



Following literature procedures,[117] thionyl chloride (1.8 mL, 25 mmol) was added dropwise to a solution of 4-iodobenzoic acid (**4-9**) (2.90 g, 11.7 mmol) in ethanol (30 mL). The solution was heated at 60 °C for 4 h, and the reaction mixture was concentrated in vacuo. The residue was diluted with EtOAc and the organic layer washed with H_2O and brine. After drying ($MgSO_4$) and concentration under reduced pressure, the oil was purified by flash chromatography with hexane/CH_2Cl_2 (8:2) to afford pure ethyl 4-iodobenzoate **4-10** (3.2 g, 98%) as a colorless oil. Proton and carbon NMR data are identical to those reported in SDBS.[137]

4-10 Ethyl 4-iodobenzoate**:** R_f = 0.31 (hexane/ CH_2Cl_2, 7:3); ^{1}H NMR ($CDCl_3$) δ 7.78-7.68 (m, 4H), 4.35 (q, J = 7.1, 2H), 1.35 (t, J = 7.1, 3H); ^{13}C NMR ($CDCl_3$) δ 166.07, 137.71, 131.07, 130.01, 100.69, 61.27, 14.41.

4-allyl benzoate (**4-11**)

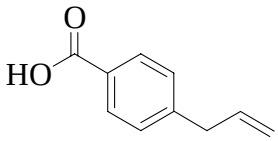


Following literature procedures,[117] isopropylmagnesium chloride (8.1 mL, 2 *M* THF solution) was added dropwise to a solution of ethyl 4-iodobenzoate (**4-10**) (3.02 g, 10.9 mmol) in dry THF (115 mL), cooled to -40 °C. The reaction was stirred for 1 h

before adding a daily prepared CuCN, 2LiCl solution in dry THF. To prepare the solution, anhydrous LiCl (0.977 g, 23.1 mmol) was first dried in a flask heated with an oil bath at 130 °C under vacuum for 2 hr. Upon cooling to room temperature, CuCN (0.990 g, 11.1 mmol) and dry THF (37 mL) were added and the flask flushed with argon. After stirring for 15 min, the yellow-green solution was cooled to -40 °C and added to the solution of ethyl 4-iodobenzoate via a stainless steel cannula. The reaction was stirred for 15 min followed by the addition of allyl bromide (3.8 mL, 44 mmol). After being stirred for 1 h, the reaction mixture was diluted with EtOAc and filtered over Celite. The filtrate was concentrated under reduced pressure and the residue redissolved in EtOAc. The organic layer was washed with H_2O and brine, dried ($MgSO_4$) and concentrated in vacuo. Purification by flashed chromatography with hexane/CH_2Cl_2 (85:15) afforded pure ethyl 4-allylbenzoate **4-11** (1.9 g, 89%) as a yellow oil.

4-11 4-allylbenzoate R_f = 0.32 (hexane/ CH_2Cl_2, 7:3); ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 8.6 Hz, 2H), 7.21 (d, J = 8.6 Hz, 2H), 5.99-5.85 (m, 1H), 5.11-5.03 (m, 2H), 4.34 (q, J = 7.1 Hz, 2H), 3.39 (d, J = 6.7 Hz, 2H), 1.36 (t, J = 7.13 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 166.41, 145.28, 136.40, 129.69, 128.71, 128.52, 116.45, 60.69, 40.08, 14.30.

4-allyl benzoic acid (**4-5**)



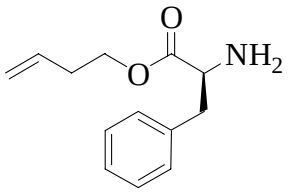
An aqueous solution of sodium hydroxide (2M, 40 mL) was added to an ethanolic solution of ethyl-4-allylbenzoate (**4-11**) (55 mM, 80 mL). The solution was stirred at r.t. for 7 h, then quenched with HCl (1 M, 80 mL). Ethanol was removed in vacuo and the aqueous layer was extracted 3x with EtOAc. The organic layer was washed with brine, dried (MgSO4), and concentrated under reduced pressure. Recrystallizing with

hexane/Et_2O afforded 4-allybenzoic acid (**4-5**) as a white solid (0.67 g, 94%). Proton and carbon NMR data are those reported in literature[138].

4-5: 4-allylbenzoic acid**:** R_f = 0.25 (CH_2Cl_2/MeOH, 95:5); ^{1}H NMR ($CDCl_3$) δ 8.04 (d, J = 8.32, 2H), 7.28 (d, J = 8.31, 2H), 6.02-5.88 (m, 1H), 5.15-5.05 (m, 2H), 3.45 (d, J = 6.66, 2H); ^{13}C NMR ($CDCl_3$) δ 172.76, 146.79, 136.43, 130.64, 128.95, 127.48, 116.94, 40.42.

6.2.7 General procedure for removing Boc protecting group with TFA

Deprotected homoallyl phenylalanine **4-12**

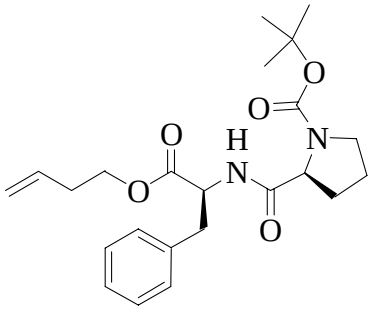


A solution of trifluoroacetic acid (4.0 mL) and CH_2Cl_2 (10 mL) was added drop-wise to a stirred solution of amino acid **3-26e** (2.35 g, 7.36 mmol) and CH_2Cl_2 (10 mL). After stirring for 3.5 h, the solvent and excess TFA were removed *in vacuo*, followed by neutralization with $NaHCO_3$, and extraction with EtOAc. The organic layer was washed with brine and dried (Na_2SO_4). Removal of the solvent followed by recrystallization in Et_2O/Hexane afforded a white solid, m.p. = 77-78 °C.

4-12: R_f = 0.34 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 7.99 (s, 2H), 7.48-7.32 (m, 5H), 5.75 (ddt, J = 18.0, 10.2, 6.7 Hz, 1H), 5.23-5.14 (m, 2H), 4.40-4.18 (m, 3H), 3.42 (dd, J = 14.2, 6.5 Hz, 1H), 3.34 (dd, J = 14.7, 7.2 Hz, 1H), 2.43-2.35 (m, 2H); ^{13}C NMR (CDCl3) δ169.24, 133.94, 133.25, 129.51, 129.14, 127.94, 117.86, 65.66, 54.33, 36.62, 32.62 .

6.2.8 General Procedure for EDCI coupling

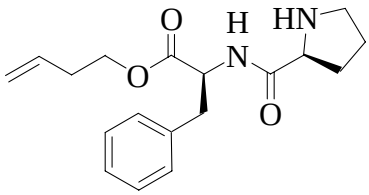
Compound **4-13**



A flame dried flask under argon was charged with acid (1.69 g, 7.83 mmol) and CH_2Cl_2 (18 mL). The reaction flask was cooled to 0 °C and EDCI (1.75 g, 9.13 mmol), HOBt (1.15 g, 8.49 mmol), DMAP (0.591 g, 0.484 mmol), and DIPEA (2.1mL, 12 mmol) were added to the stirred solution. After stirring for 30 min, amino acid **4-12** (1.43 g, 6.52 mmol) was added and the reaction maintained for 4 h at room temperature. The solvent was removed *in vacuo*, and the residue redissolved in EtOAc. The organic layer was washed with $KHSO_4$ (1 *N*), $NaHCO_3$ (1 *N*), water, and brine. The organic layer was then dried with Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography with hexane/Et_2O (7:3) afforded **4-13** (2.4 g, 85%).

4-13: R_f = 0.29 (Et_2O/hexane, 6:4); ^1H NMR ($CDCl_3$) δ 7.24-7.02 (m, 6H), 5.67 (ddt, J = 17.4, 10.3 6.7 Hz, 1H), 5.08-4.96 (m, 2H), 4.77 (s, 1H), 4.24-4.02 (m, 3H), 3.34-2.89 (m, 4H), 2.34-1.67 (m, 6H), 1.35 (s, 9H); IR (neat) 3315, 3065, 2977, 1743, 1698, 1521, 1394, 1165, 1122, 1088 cm^{-1}.

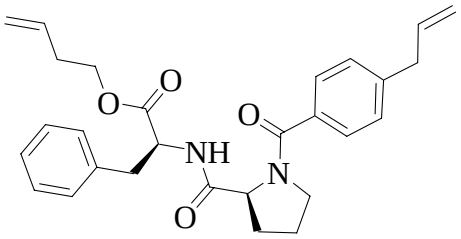
Compound **4-6**



Following general deprotection procedures using TFA, amino acid derivative (1.99 g, 4.78 mmol), TFA (4.0 mL), and CH_2Cl_2 (22 mL) gave a yellow oil. Column chromatography on silica gel with CH_2Cl_2/MeOH (97:3) yielded **4-6** as a white solid (1.5 g, 98%), m.p. = 88-90 °C.

4-6: R_f = 0.31 (CH_2Cl_2/MeOH, 95:5); ^{1}H NMR ($CDCl_3$) 8.18-8.08 (m, 2H), 7.27-7.11 (m, 5H), 5.70 (ddt, J = 17.0, 10.3, 6.7 Hz, 1H), 5.13-5.03 (m, 2H), 4.75-4.47 (m, 2H), 4.19-4.06 (m, 2H), 3.35-2.97 (m, 4H), 2.38-1.82 (m, 6H), δ; ^{13}C NMR ($CDCl_3$) δ 171.12, 169.13, 136.33, 133.69, 129.23, 128.70, 127.20, 117.68, 64.76, 59.50, 54.52, 46.43, 37.26, 32.91, 30.10, 24.43; IR (KBr) 3347, 3286, 3091, 2985, 1739, 1714, 1667, 1573, 1556, 1499, 1457, 1427, 1299, 1181, 1137, 1084, 1031 cm^{-1}; $[\alpha]^{25}_D$ = -27.7 ° (c = 1.16, MeOH); HRMS (ESI-FT-ICR-MS) for $[M+H]^+$: calcd 317.1860; found 317.1880.

Compound **4-4**



Following EDCI coupling procedures, acid **4-5** (336 mg, 2.07 mmol), **4-6** (243 mg, 0.768 mmol), EDCI (480 mg, 2.50 mmol), HOBt (338 mg, 2.50 mmol), DMAP (101 mg, 0.827 mmol), DIPEA (0.55 mL, 320 mmol), and CH_2Cl_2 (6 mL) yielded **4-4** (244 mg, 69%) as a white solid, m.p. = 84.5-86 °C.

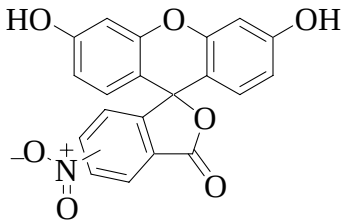
4-4: R_f = 0.33 (hexane/EtOAc, 1:1); ^{1}H NMR ($CDCl_3$) δ 7.43-7.08 (m, 10H), 6.03-5.65 (m, 2H), 5.14-4.74 (m, 6H), 4.21-4.11 (m, 2H), 3.47-2.97 (m, 6H), 2.42-1.72 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 171.43, 171.15, 171.00, 142.67, 136.79, 136.30, 133.99,

133.77, 129.48, 128.60, 128.54, 127.61, 126.99, 117.60, 116.53, 64.52, 59.91, 53.65, 50.49, 40.13, 38.08, 32.98, 27.43, 25.46zz; IR (KBr) 3258, 3078, 2978, 1733, 1633, 1539, 1423, 1351, 1324, 1272, 1228, 1178, 1118, 1083, 1043 cm^{-1}; $[\alpha]^{25}_{D}$ = - 39.4° (c = 1.10, MeOH); HRMS (ESI-FT-ICR-MS) for $[M+Na]^+$: calcd 483.2254, found 483.2258; Anal. calcd for $C_{28}H_{32}N_2O_4$: C, 73.02; H, 7.00; N, 6.08. Found C, 72.76; H, 7.15; N, 6.00%

Dimer **4-7a**

4-7a: ^{1}H NMR (Acetone d_6) δ 7.39-7.18 (m, 9H), 6.62 (d, J = 7.2 Hz, 1H), 5.84 (dtt, J = 15.3, 6.0, 1.1 Hz, 1H), 5.04 (dddt, J = 14.3, 7.9, 6.2, 1.6 Hz, 1H), 4.55 (dd, J = 8.3, 3.5 Hz, 1H), 4.28 (q, J = 7.3 Hz, 1H), 4.27 (m, 1H), 3.82 (m, 1H), 3.62 (m, 2H), 3.38 (dd, J = 15.1, 6.6 Hz, 1H), 3.22 (dd, J = 14.5, 6.2 Hz, 1H), 3.02 (dd, J = 14.1, 7.6 Hz, 1H), 2.66 (dd, J = 13.7, 7.7 Hz, 1H), 2.25 (m, 2H), 2.18 (m, 1H), 1.84 (m, 2H), 1.76 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 171.3, 169.1, 169.8, 142.6, 138.1, 132.7, 129.8, 128.4, 128.2, 126.7, 63.5, 62.1, 55.2, 47.4, 37.9, 37.3, 32.4, 32.2, 22.9; HRMS (ESI-FT-ICR-MS) for $[M+H]^+$: calcd 865.4171, found 865.4174.

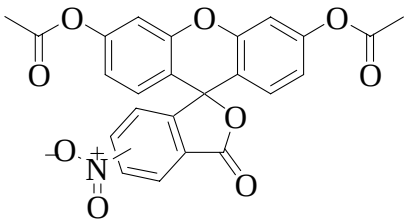
5,6-nitrofluorescein (**5-20**)



Following literature procedures, resorcinol (**5-18**) (26.7 g, 243 mmol) and 4-nitrophthalic acid (**5-19**) (25.5 g, 121 mmol) were thoroughly mixed in a flask and heated on an oil bath at 190-200 °C until the black-brown liquid dried to a glass-like mass (*ca.*12 hours). After cooling to room temperature, the melt was chipped from the flask, ground

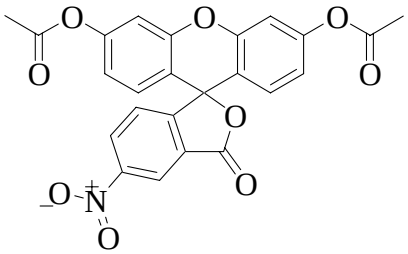
in a mortar, and suspended in 0.6 *N* HCl (400 mL). The mixture was boiled for 1 hour, filtered hot in a 350 mL coarse fritted filter, washed with hot 0.6 *N* HCl (3 x 75 mL), followed with 1.5 L of boiling water. The brown mass was dried in the oven at 112 °C to give 5,6-nitrofluorescein (**5-20**) (42 g, 94%), m.p. 338-340 °C. R_f = 0.41 (benzene/EtOH, 4:1).

5,6-nitrofluorescein diacetate (**5-21**)



To a stirred solution of 5,6-nitrofluorescein (**5-20**) (35.5 g, 76.9 mmol) in pyridine (136 mL) at room temperature was added glacial acetic anhydride (53 mL). Acetylation occurred immediately and no changes in TLC were observed after 10 minutes. Three volumes of toluene were added to the solution, stirred for 5 minutes, and concentrated under reduced pressure to form a black-brown viscous gum. After repeating this step two more times, the viscous mass was vacuum dried to form 5,6-nitrofluorescein diacetate **5-21**, 100 °C decomposition. R_f = 0.70 (benzene/EtOH, 4:1).

5-nitrofluorescein diacetate (**5-22**)

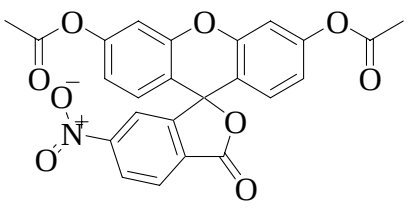


Following literature procedures, 5,6-nitrofluorescein diacetate **5-21** was redissolved in 100 mL refluxing acetic anhydride, cooled to room temperature, seeded with 5-nitrofluorescein diacetate crystal previously obtained to induce crystallization, and set

overnight. Recrystallization continued for another day at 4 °C. The creamy yellow-white crystals formed were collected in a Buchner funnel, and washed twice with 35 mL of acetic anhydride (6.3 g, 14%, fraction 1). The filtrate from fraction 1 was concentrated to half the volume under reduced pressure, seeded with crystals, and allowed to crystallize at 4 °C for 48 hours to obtain additional 5-nitrofluorescein diacetate. The crystals were collected in a Buchner funnel and rinsed twice with 35 mL of acetic anhydride (9.3 g, 21%, fraction 2). To purify the 5-nitrofluorescein diacetate, fractions 1 and 2 were combined, dissolved with 50 mL refluxing acetic anhydride, and filtered hot on a Buchner funnel. The filtrate was cooled to 4 °C for 24 hours. The white crystals formed were collected in a Buchner funnel and rinsed twice with 20 mL acetic anhydride to give purified 5-nitrofluorescein diacetate **5-22** (9.0 g, 21%). To increase the yield, the filtrate from the purification of fraction 1 and 2 was concentrated under reduced pressure, and cooled at 4◦C for 24 hours. The crystals formed in the flask were isolated and purified as described above (1.1 g, 3%, fraction 3). Total yield of all fractions combined after purification was 10.1 g (24%), m.p. 223-224 °C.

5-22: R_f = 0.71 (benzene/EtOH, 4:1); ^{1}H NMR ($CDCl_3$) δ 8.88 (d, J = 1.9 Hz, 1H), 8.55 (dd, J = 2.0, 8.4 Hz, 2H), 7.40 (d, J = 8.5 Hz, 1H), 7.15 (d, J = 1.9 Hz, 2H), 6.90-6.79 (m, 4H), 2.34 (s, 6H) ; ^{13}C NMR ($CDCl_3$) 168.93, 166.78, 157.87, 152.67, 151.54, 149.67, 130.29, 128.80, 127.71, 125.75, 121.20, 118.34, 114.98, 110.96, 82.22, 21.25; HRMS for $C_{20}H_{25}NO_9$ $[M]^+$, calcd 461.0747, found 461.0747. Melting point is an agreement with literature.[129]

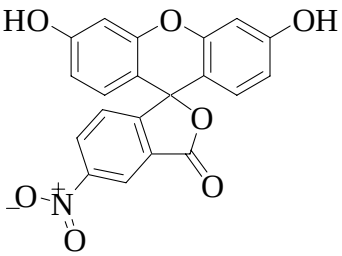
6-nitrofluorescein diacetate (**5-23**)



The filtrate from fraction 2 of **5-22** was concentrated under reduced pressure to form a dark brown viscous gum. The mass was redissolved in 180 mL of benzene and recrystallized overnight at room temperature. The yellow-white crystals were collected in a Buchner and washed with 40 mL of benzene (5.1 g, 12%), m.p. 195 °C. If higher yields were required, the filtrate was concentrated under vacuum and recrystallized at 4 °C in benzene.

5-23: R_f = 0.69 (benzene/EtOH ,4:1); ^{1}H NMR ($CDCl_3$) δ 8.49 (dd, J = 1.8, 8.5 Hz, 1H), 8.21 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 1.7Hz, 1H), 7.15 (d, J = 2.1Hz, 2H), 6.90-6.79 (m, 4H), 2.33 (s, 6H); ^{13}C ($CDCl_3$) 168.87, 166.97, 154.26, 152.69, 151.65, 130.77, 128.75, 125.50, 126.90, 125.76, 119.84, 118.37, 114.98, 110.99, 82.22, 21.28. Melting point is an agreement with known literature.[129]

5-nitrofluorescein (**5-24**)

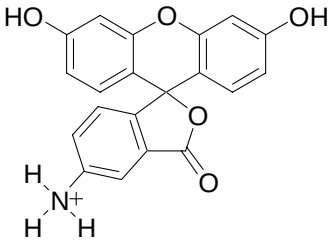


5-nitrofluorescein diacetate **5-22** (4.75 g, 10.3 mmol) was dissolved in hot filtered methanol saturated with NaOH (95 mL), and stirred at 50 °C. The dark-red solution was filtered on a Buchner funnel, poured into 4 volumes of water, and acidified with 12.1 *N* HCl (2 mL). The orange mixture was allowed to sit at room temperature for 4 hours.

The orange precipitate was collected in a Buchner funnel, washed with water (480 mL), and vacuum dried to give **5-24** (3.8 g, 97 %), m.p. above 350 °C.

5-24: R_f = 0.40 (benzene/EtOH, 4:1); IR (KBr) 3064.97, 1599.09, 1528.31, 1459.81, 1353.42, 1311.36, 1240.52 1212.21, 1172.09, 1120.48 cm^{-1}; ^1H NMR δ 10.22 (s, 2H), 8.66 (d, J = 2.0Hz, 1H), 8.56 (dd, J = 2.2, 8.4 Hz, 1H), 7.57 (d, 8.4 Hz, 1H), 6.72-6.52 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 166.74, 159.88, 157.40, 151.83, 149.10, 130.40, 129.41, 127.74, 125.93, 120.35, 112.78, 108.29, 102.38, 83.77; HRMS for $C_{20}H_{11}NO_7$ $[M]^+$, calcd 377.0536, found 377.0531; Melting point is in agreement with known literature.[129] IR is in agreement with SDBS.[137]

5-aminofluorescein salt (**5-25**)

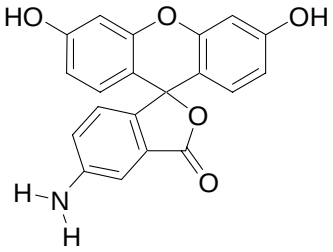


To a stirred solution of sodium sulfide nonahydrate, $Na_2S•9H_2O$, (4.35 g, 18.1 mmol), in H_2O (75 mL) was added 5-nitrofluorescein (**5-24**) (1.89 g, 5.01 mmol). After it was completely dissolved, sodium hydrosulfide, NaHS (2.04 g, 36.4 mmol), was introduced and refluxed for 24 h at 125 °C. The solution was cooled to room temperature and acidified with glacial acetic acid (3.0 mL). The dark red precipitate was collected in a Buchner funnel, dissolved in refluxing 6% HCl (150 mL), and filtered hot in a Buchner funnel to remove elemental sulfur. The filtrate was cooled to room temperature then placed at 4 °C for 48 hours allowing crystallization. The red-orange crystals were collected in a Buchner funnel, redissolved in refluxing 6% HCl (50 mL), and filtered hot

in a Buchner funnel to remove any additional sulfur. Crystallization at 4 °C for 48 h gave 5-aminofluorescein salt (**5-25**) (0.88 g, 46%), 204 °C decomposition.

5-25: R_f = 0.26 (benzene/EtOH, 4:1); ^{1}H NMR (DMSO-d_6) δ 7.56 (s, 1H), 7.41 (d, J = 8.3 Hz, 1H), 7.18 (d, J = 8.4 Hz, 1H), 6.77-6.55 (m, 6H).

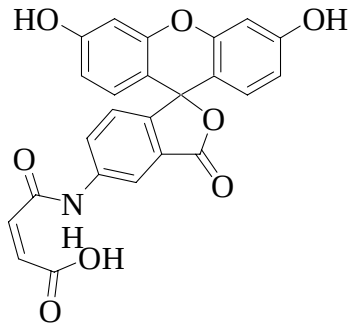
5-aminofluorescein (**5-26**)



5-nitrofluorescein salt (**5-25**) (425 mg, 1.11 mmol) in NaOH (88 mL) was precipitated with glacial acetic acid (2.5 mL). The solid was collected in a Buchner funnel, and dried under vacuum to give **5-26** (270 mg, 67%), m.p. 222 °C.

5-26: R_f = 0.58 (benzene/EtOH, 3:2); ^{1}H NMR (DMSO-d_6) δ 10.97 (s, 2H), 7.00-6.82 (m, 3H), 6.67-6.50 (m, 6H), 5.75 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 169.55, 159.35, 152.05, 150.58, 139.72, 129.12, 127.58, 124.27, 121.77, 112.50, 110.77, 106.24, 102.19, 82.96; HRMS for $C_{20}H_{13}NO_5$ $[M]^+$, calcd 347.0794, found 347.0793. Spectral data consistent with sample of 5-aminofluorescein purchased from Aldrich and SDBS.[137]

N-(5-Fluoresceinyl)maleamic acid (**5-28**)

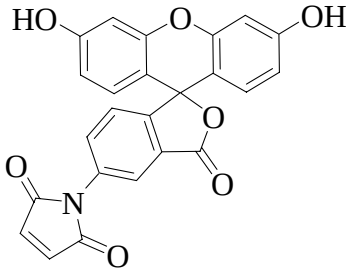


Maleic anhydride (**5-27**) (49.6 mg, 0.506 mmol) was added to a stirred solution of 5-aminofluorescein (**5-26**) (174 mg, 0.500 mmol) in glacial acetic acid (50 mL). The

mixture was stirred for 4 h at room temperature. The bright canary yellow precipitate was collected on a filtered frit, washed with EtOAc (100 mL), dried overnight, and used in the next step without purification (190 mg, 85%), m.p. above 350 °C.

5-28: R_f = 0.10 (benzene/EtOH, 60:40,); 1H NMR (DMSO-d_6) δ 12.93 (br, 1H), 10.77 (s, 2H), 10.13 (s, 2H), 8.32 (s, 1H), 7.84 (dd, J = 1.8, 8.4 Hz, 1H), 7.24 (d, J = 8.4 Hz, 1H), 6.51-6.68 (m, 7H), 6.34 (d, J = 12.0 Hz, 1H). All data is in agreement with known literature.[127]

N-(5-Fluoresceinyl)maleimide (**5-13**)

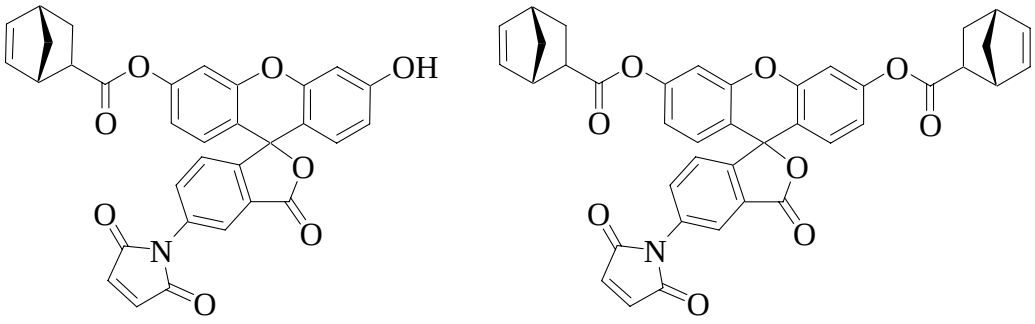


To a stirred solution of maleic acid **5-28** (161 mg, 0.25 mmol) in anhydrous DMF (1.5 mL) was added distilled benzene and $ZnCl_2$ (68.2 mg, 0.500 mmol). Freshly distilled HMDS (211 ul) was added dropwise, and the mixture refluxed for 2.5 h. After cooling to room temperature, the mixture was filtered and the orange-yellow filtrate collected. The solvent was removed under reduced pressure, leaving behind DMF and the maleimide. The residual DMF was poured into an ice-water bath (50 mL), and the aqueous phase acidified with 0.1 *N* HCl to a pH of 6. The orange-yellow precipitate was collected in a filtered frit and dried in a dessicator under vacuum in the dark to give maleimide **5-13** (97 mg, 91%), m.p. above 350 °C.

5-13: R_f = 0.64 (benzene/EtOH, 3:2); ^{1}H NMR (DMSO-d_6) 10.18 (s, 2H), 7.98 (d, J = 1.2 Hz, 1H), 7.79 (dd, J = 1.7, 8.2 Hz, 1H), 7.42 (d, J = 8.2 Hz, 1H), 7.28 (s, 2H), 6.55-6.71 (m, 6H); ^{13}C (NMR) 169.65, 168.02, 159.62, 151.83, 151.10, 134.96, 133.50,

133.20, 129.14, 126.73, 124.72, 122.02, 112.76, 109.17, 102.32, 83.34; HRMS for $C_{24}H_{13}NO_7$ [M]+, calcd 427.0692 found 427.0685. All data is an agreement with known literature.[127]

Mono-Norbornene Ester (5-Fluoresceinyl)maleimide (**5-12**) and Di-Norbornene Ester (5-Fluoresceinyl)maleimide (**5-14**)

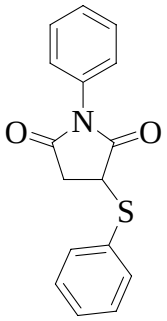


Under argon, oxalyl chloride (510 µl, 5.80 mmol) was added dropwise to a stirred solution of 5-norbornene-2-carboxylic acid (**5-9**) (98%, mixture of endo and exo, 160 mg, 1.16 mmol) and CH_2Cl_2 (1.2 mL). The mixture was stirred until no additional gas was released. Excess oxalyl chloride was removed under reduced pressure. To remove any oxalyl chloride residue, the acid chloride was redissolved in CH_2Cl_2 and concentrated under reduced pressure. The crude acid chloride was redissolved in CH_2Cl_2 (7.0 mL) once more, and added dropwise to a stirred solution of maleimide **5-13** (472 mg, 1.10 mmol) in triethylamine (200 ul, 1.44 mmol) and CH_2Cl_2 (34 mL) at 0 °C. The reaction was allowed to warm to room temperature and stirred for 3 h. The yellow orange mixture was diluted with CH_2Cl_2, extracted from brine, and dried (Na_2SO_4). Concentration by reduced pressure and purification by flash chromatography on silica gel with CH_2Cl_2/MeOH (100:0 to 98:2) gave **5-12** (mixture of endo and exo) as a yellow-orange solid (130 mg, 22%), 183 °C decomposition, and di-acylated **5-14** (mixture of endo and exo) (40 mg, 6%) as a white yellow precipitate, m.p. 205.5-207◦C.

5-12: R_f = 0.38 (CH_2Cl_2/MeOH, 96:4); ^{1}H NMR ($CDCl_3$) δ 8.05 (d, J = 1.6 Hz, 1H), 7.67 (dd, J = 1.5, 8.3 Hz, 1H), 7.22-6.43 (m, 9H), 6.28 (dt, J = 2.8, 5.6 Hz, 1H), 6.07 (td, J = 2.8, 5.9Hz, 1H), 3.39 (s, 1H), 3.24 (dt, J = 4.5, 8.9Hz, 1H), 2.98 (s, 1H), 2.02 (ddd, J = 4.1, 9.2, 12.4 Hz, 1H), 1.65-1.22 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 173.60, 169.08, 168.86, 158.76, 152.40, 152.26, 152.00, 151.83, 147.41, 138.69, 134.69, 133.15, 132.53, 132.16, 129.38, 127.61, 125.09, 122.20, 117.63, 116.07, 112.95, 110.66, 109.92, 103.28, 83.35, 49.98, 47.05, 46.21, 43.86, 42.87, 36.98, 29.55; IR (KBr) 3423.45, 2976.13, 1761.01, 1719.81, 1611.55, 1496.32, 1426.63, 1388.56, 1251.64, 1149.70; HRMS for $C_{32}H_{21}NO_8$ $[M]^+$,; calcd 547.1267, found 547.1254.

Di-acylated **5-14**: R_f = 0.76 (CH_2Cl_2/MeOH, 96:4); ^{1}H NMR ($CDCl_3$) δ 8.00 (d, J = 2.1 Hz, 1H), 7.63 (dd, J = 2.1, 8.3 Hz, 1H), 7.17 (d, J = 8.0 Hz, 1H), 6.96 (dd, J = 3.4, 2.2 Hz, 2H), 6.86 (s, 2H), 6.81-6.76 (m, 2H), 6.73-6.68 (m, 2H), 6.20 (dt, J = 3.0, 6.0 Hz, 2H), 5.98 (td, J=2.8, 6.0 Hz, 2H), 3.31 (s, 2H), 3.18-3.11 (m, 2H), 2.91 (s, 2H), 1.99-1.90 (m, 2H), 1.54-1.26 (m, 6H); ^{13}C NMR ($CDCl_3$) 174.44, 172.85, 168.96, 168.19, 152.62, 151.63, 147.37, 138.56, 135.70, 134.66, 133.31, 132.43, 132.14, 130.28, 129.13, 127.29, 124.98, 122.20, 118.01, 115.78, 110.56, 82.02, 49.91, 46.98, 46.52, 46.12, 43.80, 43.48, 42.82, 41.90, 30.73, 29.50; HRMS for $C_{40}H_{29}NO_9$ $[M]^+$, calcd 667.1842, found 667.1857; IR (KBr) 3481.93, 3061.02, 1975.28, 2873.30, 1766.18, 1720.31, 1610.77, 1580.32, 1495.13, 1421.42, 1386.41, 1335.54, 1244.58, 1154.72, 1015.77, 903.43, 832.28, 710.82, 690.26, 607.50.

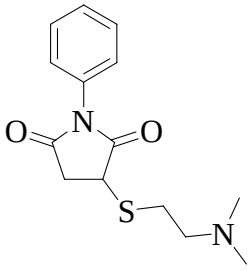
N-Phenyl-2-(phenylthio)succinimide (**5-31**)



To a stirred solution of N-phenylmaleimide (**5-29**) (3.0 g, 17.3 mmol) in dry benzene (30 mL) and triethylamine (0.17 mL) was added dropwise over 25 min a solution of benzenethiol (**5-30**) (1.8 mL, 17.5 mmol) in benzene (30 mL). Within 15 minutes a white precipitate was formed. The creamy white mixture was diluted with benzene (20 mL) and stirred overnight. The solvent was removed under reduced pressure and recrystallized in petroleum/ethyl acetate (5:1) to give **5-31** (4.9 g, 90%) m.p.140-142 °C.

5-31: R_f = 0.30 (Et_2O/Hexane, 3:2); ^{1}H NMR ($CDCl_3$) δ 7.60-7.55 (m, 2H), 7.45-7.30 (m, 6H), 7.05-7.00 (m, 2H), 4.13 (dd, J = 3.9, 9.3 Hz, 1H), 3.32 (dd, J = 9.3,19.1 Hz, 1H), 2.88 (J = 3.8, Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 174.68, 173.69, 135.24, 131.64, 129.83, 129.66, 129.29, 128.95, 126.47, 44.24, 35.51. Melting point and ^{1}H NMR data are in agreement with known literature values.[133]

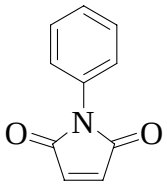
N-Phenyl-2-(dimethylamino-ethylsulfanyl)succinimide (**5-33**)



Dimethylaminoethanethiol salt (**5-32**) (2.01 g, 14.1 mmol) in Et_2O (8.0 mL) was stirred with aqueous sodium carbonate at room temperature for 20 min. The free thiol was extracted with Et_2O several times (3 x 8 mL) and dried over sodium sulfate. The solution was added dropwise for a period of 20 min to a stirred solution of N-phenylmaleimide (**5-29**) (810 mg, 4.70 mmol) in benzene (5.0 mL) and triethylamine (65 µl) at room temperature. The reaction was monitored by TLC, and after 2 h, the solvent was removed under reduced pressure. The pink white residue was recrystallized in CH_2Cl_2/hexane (1:2) to give purified **5-33** (1.1 g, 87%), m.p. 79.5-81 °C;

5-33: R_f = 0.43 (CH_2Cl_2/MeOH, 85:15); ^{1}H NMR ($CDCl_3$) δ 7.53-7.28 (m, 5H), 4.02 (J = 3.7, 9.2 Hz, 1H), 3.32 (J = 9.2, 18.7 Hz, 1H), 3.15 (J = 6.6, 13.0 Hz, 1H), 2.98 (J = 6.6, 13.0 Hz, 1H), 2.74-2.59 (m, 3H), 2.28 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 175.98, 173.93, 131.82, 129.41, 128.97, 126.61, 58.84, 45.38, 39.24, 36.25, 29.95; IR (KBr) 2946.68, 2758.14, 1707.63, 1501.56, 1455.19, 1398.60, 1300.80, 1210.37, 1187.06, 749.49, 698.93 cm^{-1}; HRMS for $C_{14}H_{18}N_2O_2S$ $[M+H]^+$, calcd 278.1089, found 279.1090;

N-Phenylmaleimide (**5-29**)



Metachloroperbenzoic acid (925mg, 5.36 mmol) in chloroform (13) was added to a stirred solution of **5-31** (1.11g, 3.92 mmol) in chloroform (20 mL) at 0 °C. The solution slowly warmed to room temperature and stirred for 1 h. The mixture was extracted with CH_2Cl_2 and washed with $NaHCO_3$ (1 *N*), H_2O, HCl (0.5 *N*), H_2O, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated under reduced pressure leaving behind a white solid. To the solid was added distilled toluene and the mixture refluxed

for 1 h. The yellow solution was cooled to room temperature and the solvent removed under reduced pressure. The yellow residue was purified by flash chromatography with silica gel (Et_2O/hexane, 2:8 to 3:7), to give maleimide **5-29** (590 mg, 87%), m.p. 86 ◦C.

5-29: R_f = 0.39 (Et_2O/hexane, 3:2); ^{1}H NMR ($CDCl_3$) δ 7.48-7.26 (m, 5H), 6.74 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 169.65, 134.32, 131.35, 129.26, 128.08, 16.21. All data is in agreement with **5-29** purchased from Aldrich.

APPENDIX A
HPLC DATA

The HPLC spectra of selected compounds from Chapter 4 are illustrated in this appendix.

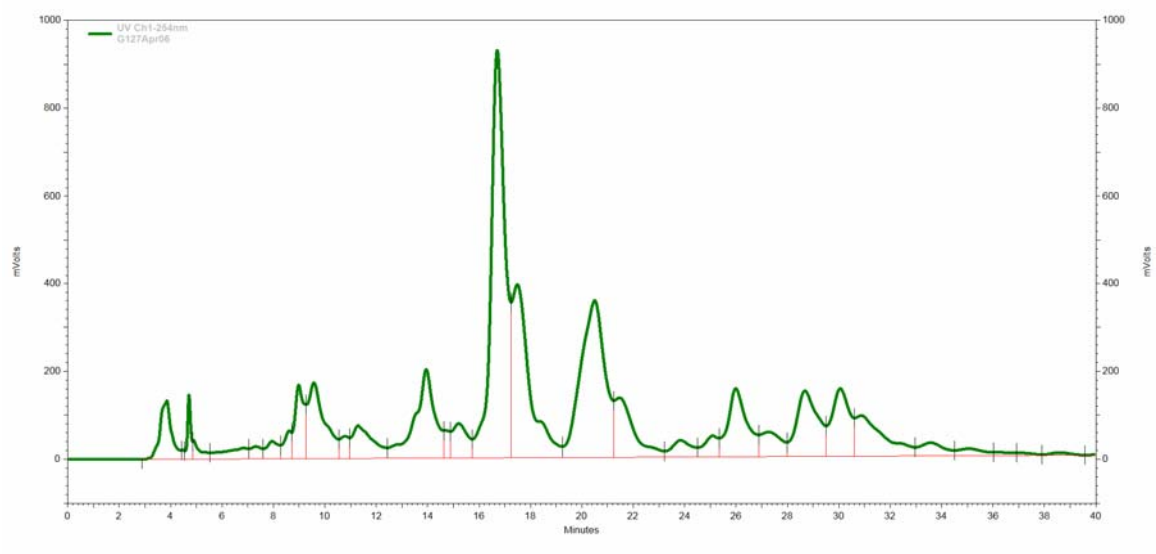


Figure A-1. HPLC spectrum of Experiment 1 reaction mixture (CH_2Cl_2, 5mM, room temperature, 4 days, no template)

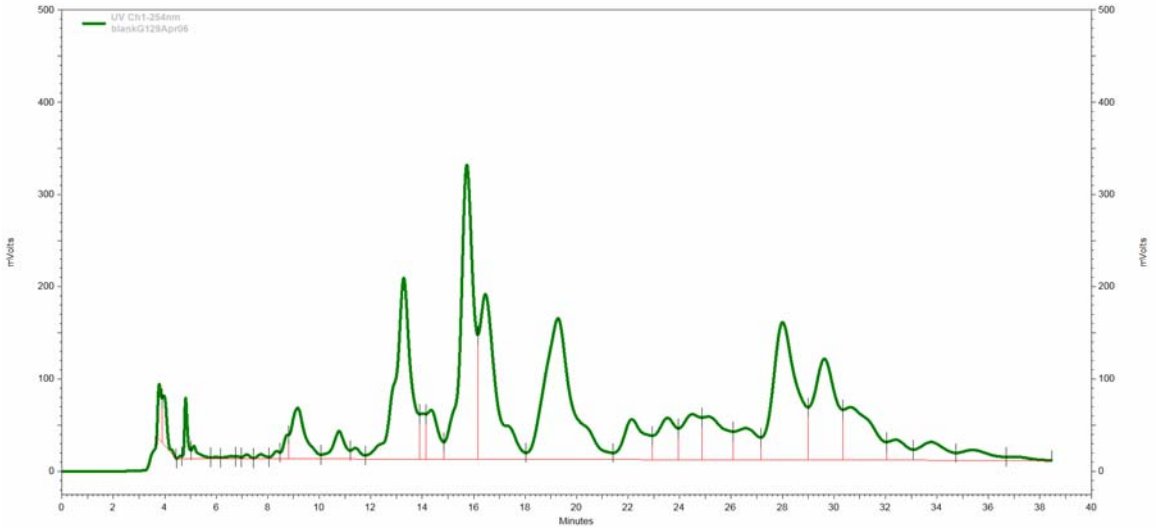


Figure A-2. HPLC spectrum of Experiment2 reaction mixture (CH_2Cl_2, 36mM, room temperature, 4 days, no template)

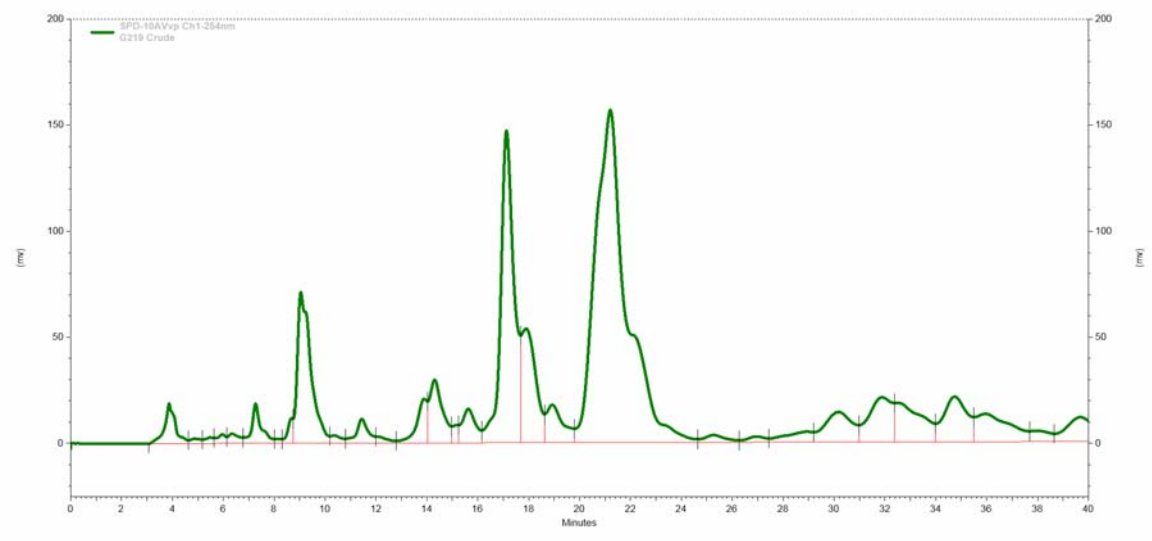


Figure A-3. HPLC spectrum of Experiment 3 reaction mixture (CH_2Cl_2, 5mM, reflux 15 hours, no template)

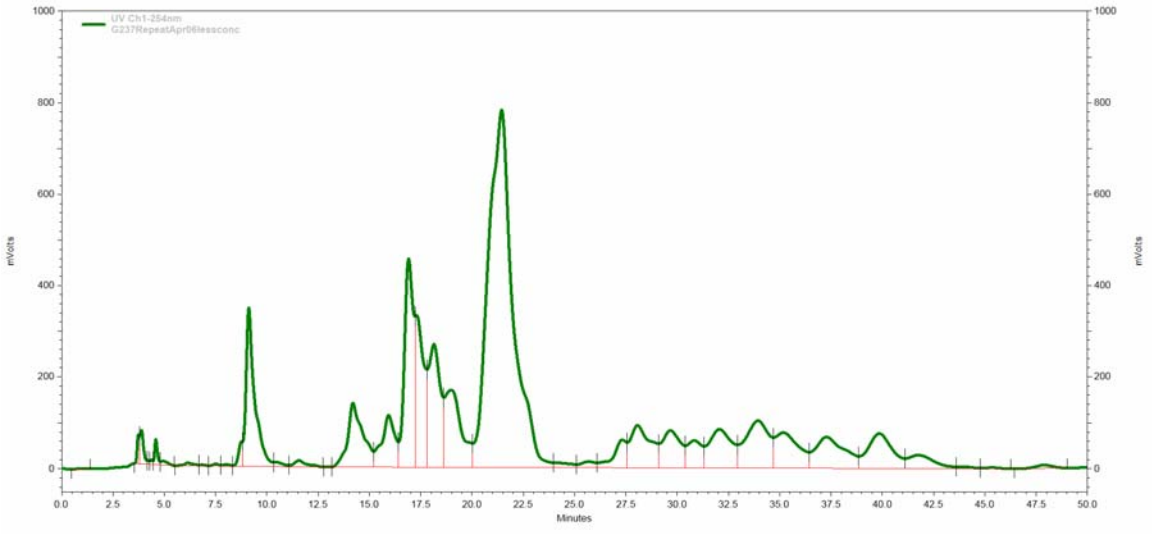


Figure A-4. HPLC spectrum of Experiment 4 reaction mixture (CH_2Cl_2, 5mM, reflux 15 hours, no template)

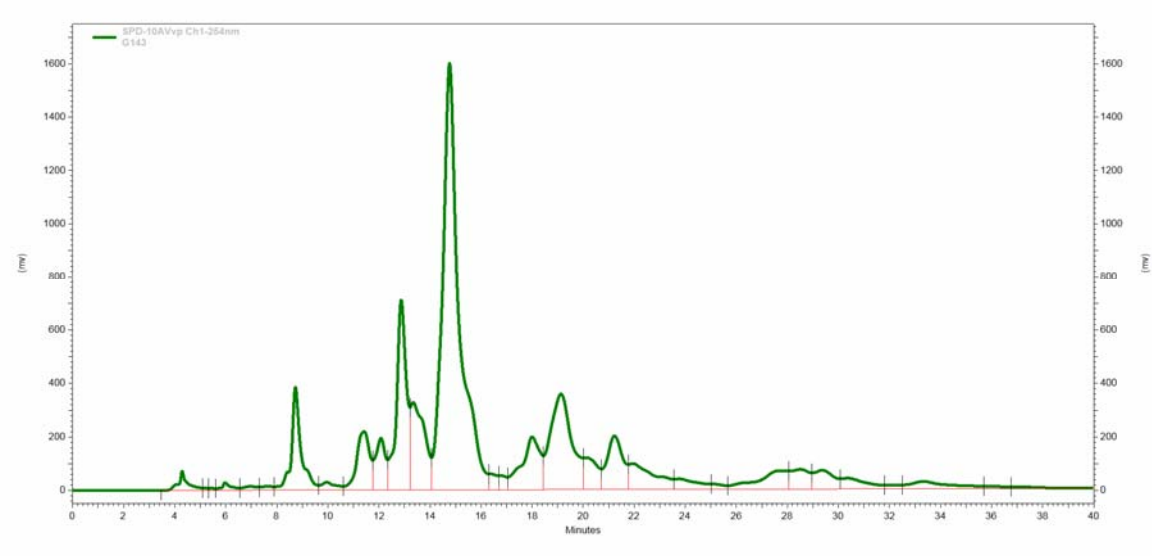


Figure A-5. HPLC spectrum of Experiment 5 reaction mixture (CH_2Cl_2, 5mM, reflux 19 hours, no template)

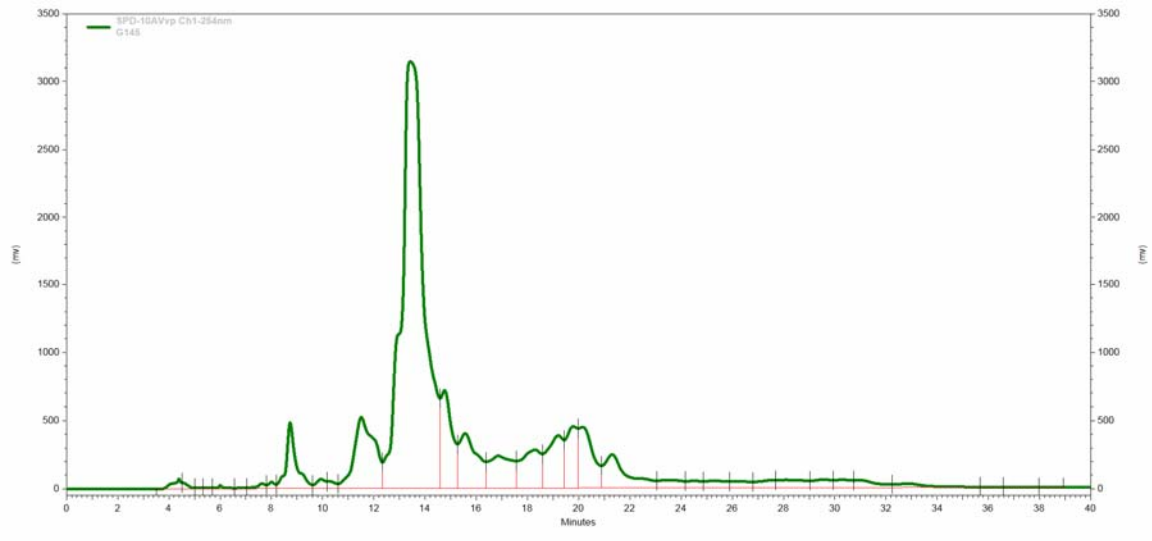


Figure A-6. HPLC spectrum of Experiment 6 reaction mixture ($CHCl_3$, 5mM, Reflux 19 hours, no template)

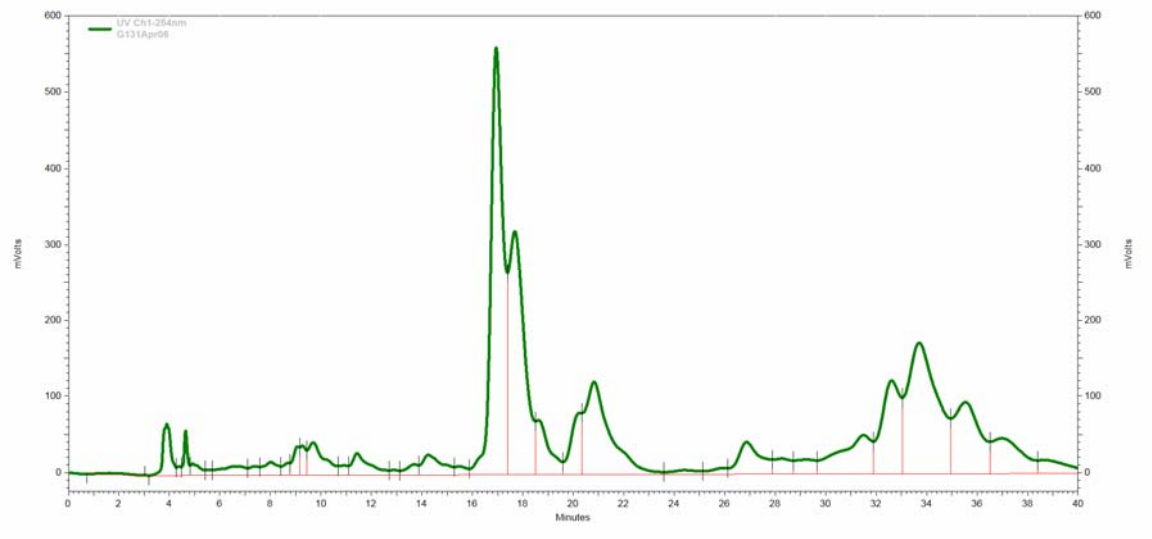


Figure A-7. HPLC spectrum of Experiment 9 reaction mixture (CH_2Cl_2, 5mM, room temperature 4 days, $LiClO_4$)

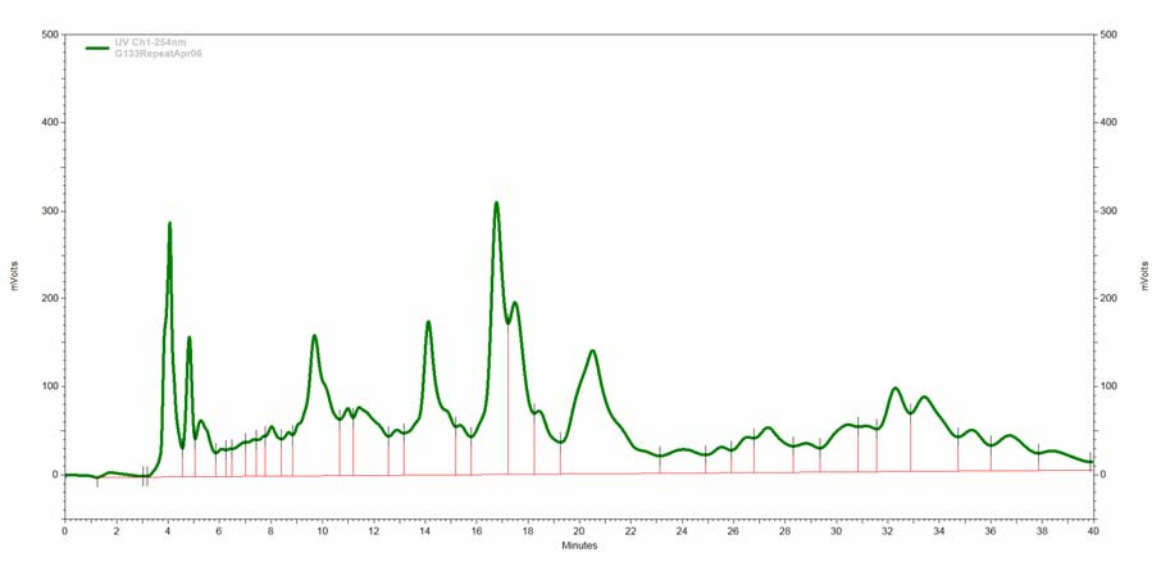


Figure A-8. HPLC spectrum of Experiment 10 reaction mixture (CH_2Cl_2, 36mM, room temperature 4 days, $LiClO_4$)

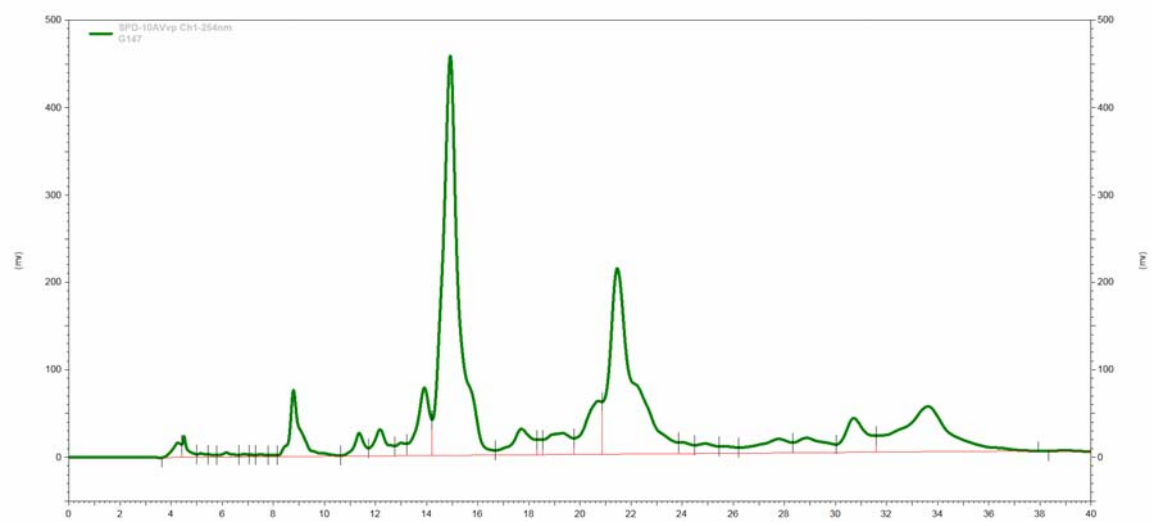


Figure A-9. HPLC spectrum of Experiment 11 reaction mixture (CH_2Cl_2, 5mM, Reflux 13 hours, LiI)

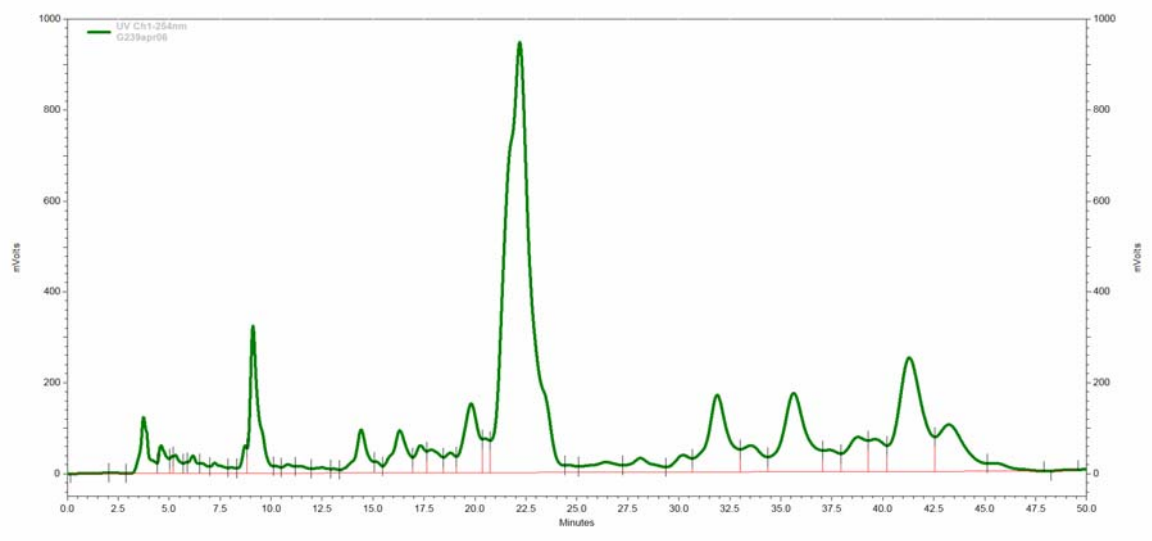


Figure A-10. HPLC spectrum of Experiment 13 reaction mixture (CH_2Cl_2, 5mM, Reflux 15 hours, LiI)

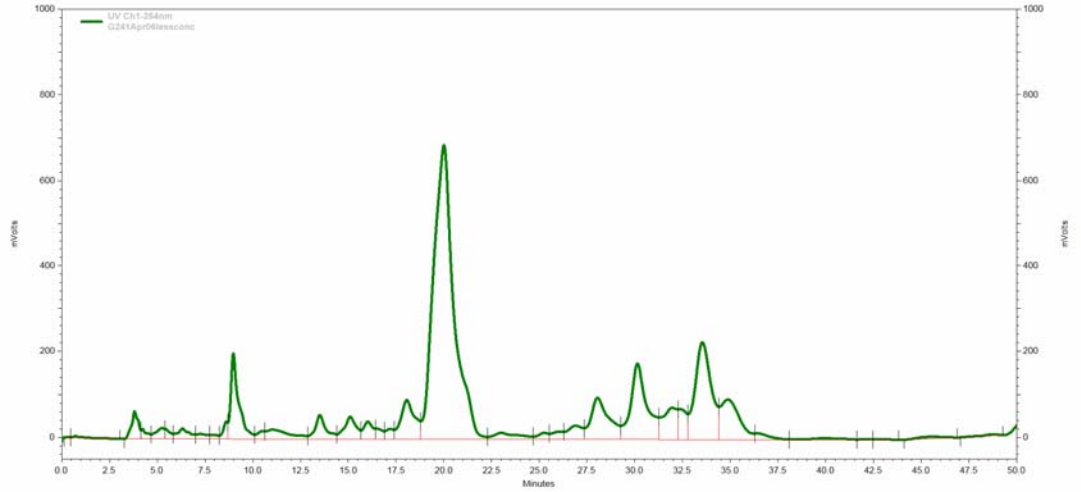


Figure A-11. HPLC spectrum of Experiment 14 reaction mixture (CH_2Cl_2, 5mM, Reflux 15 hours, LiI

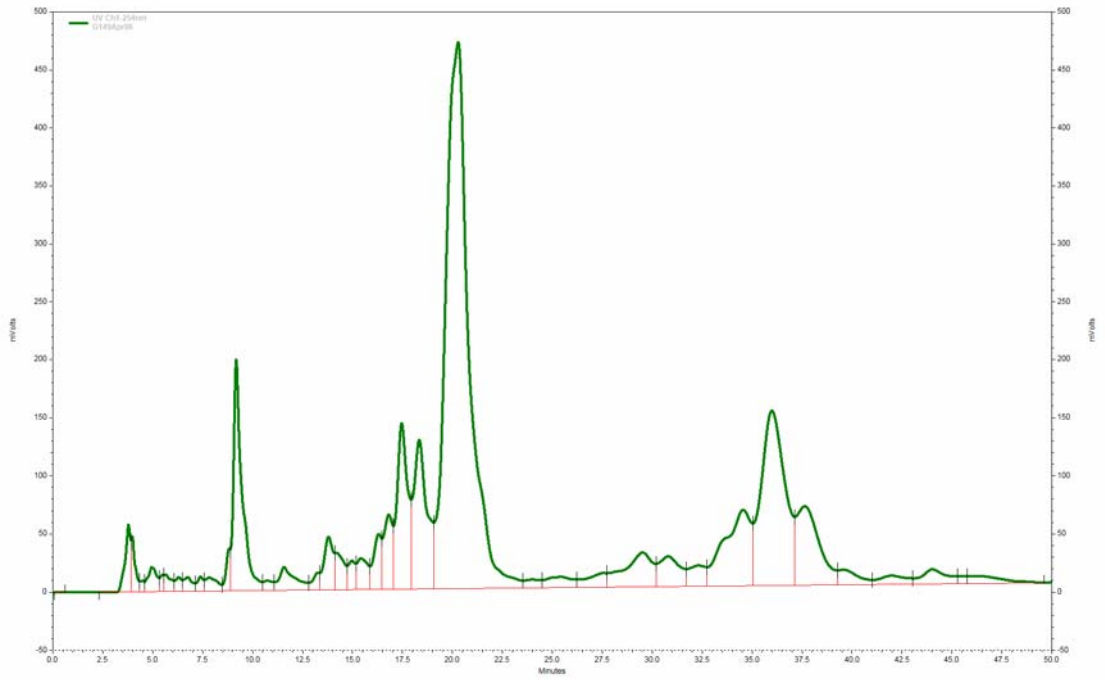
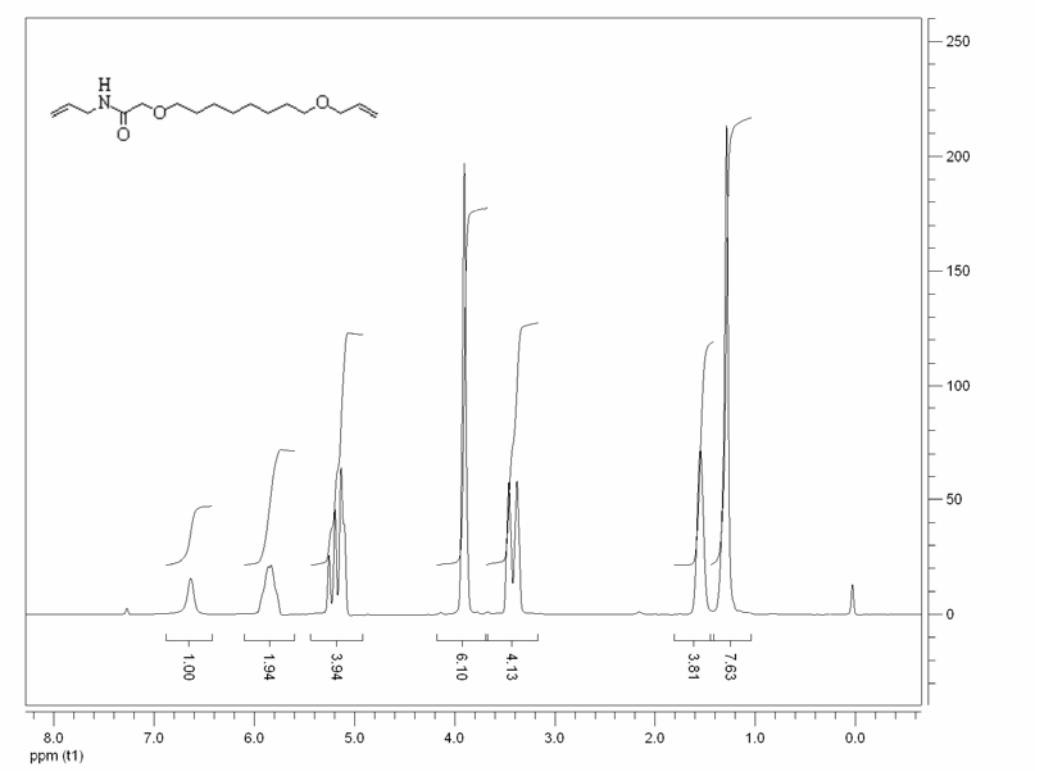
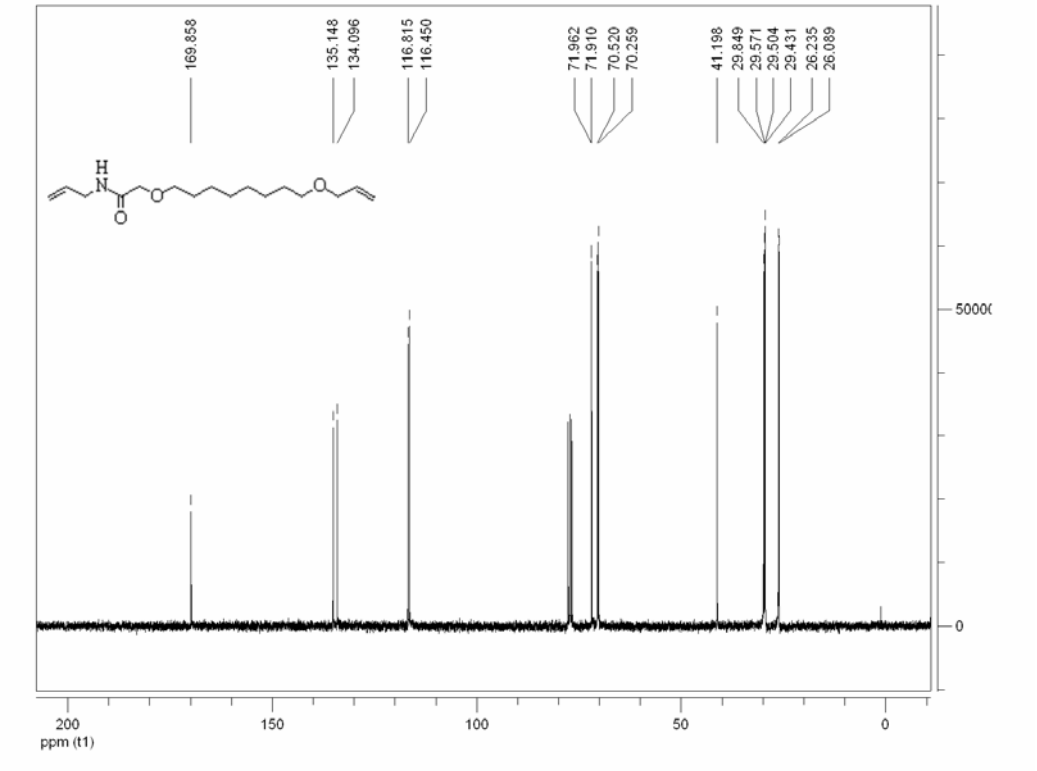


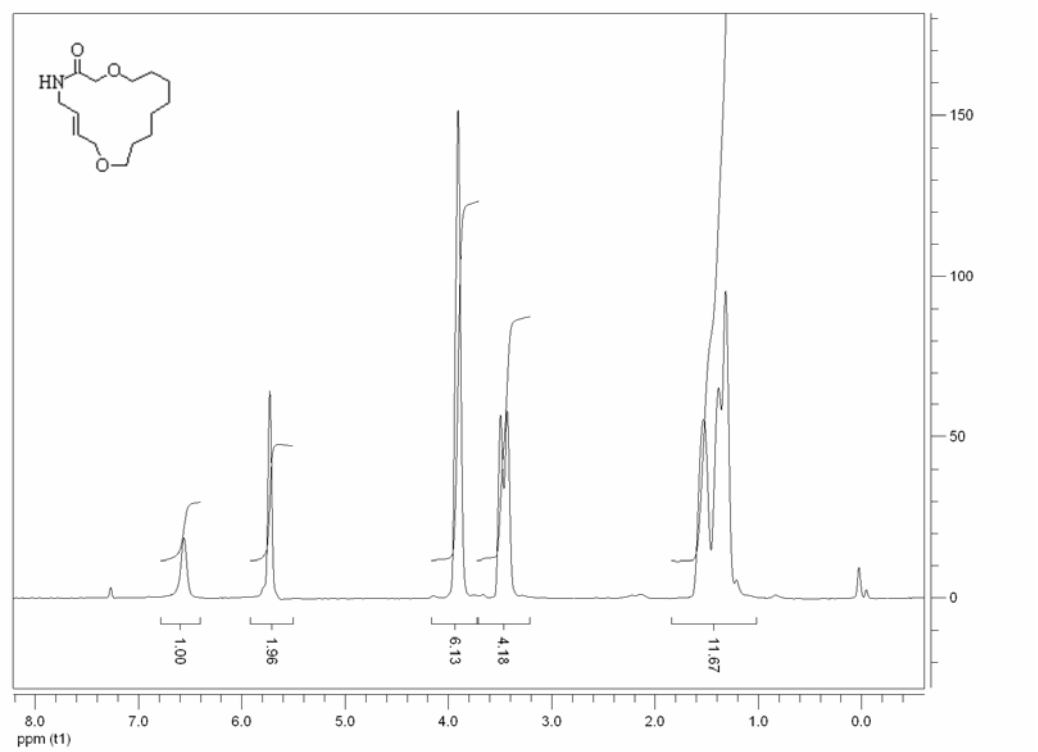
Figure A-12. HPLC spectrum of Experiment 15 reaction mixture ($CHCl_3$, 5mM, Reflux 15 hours, LiI)

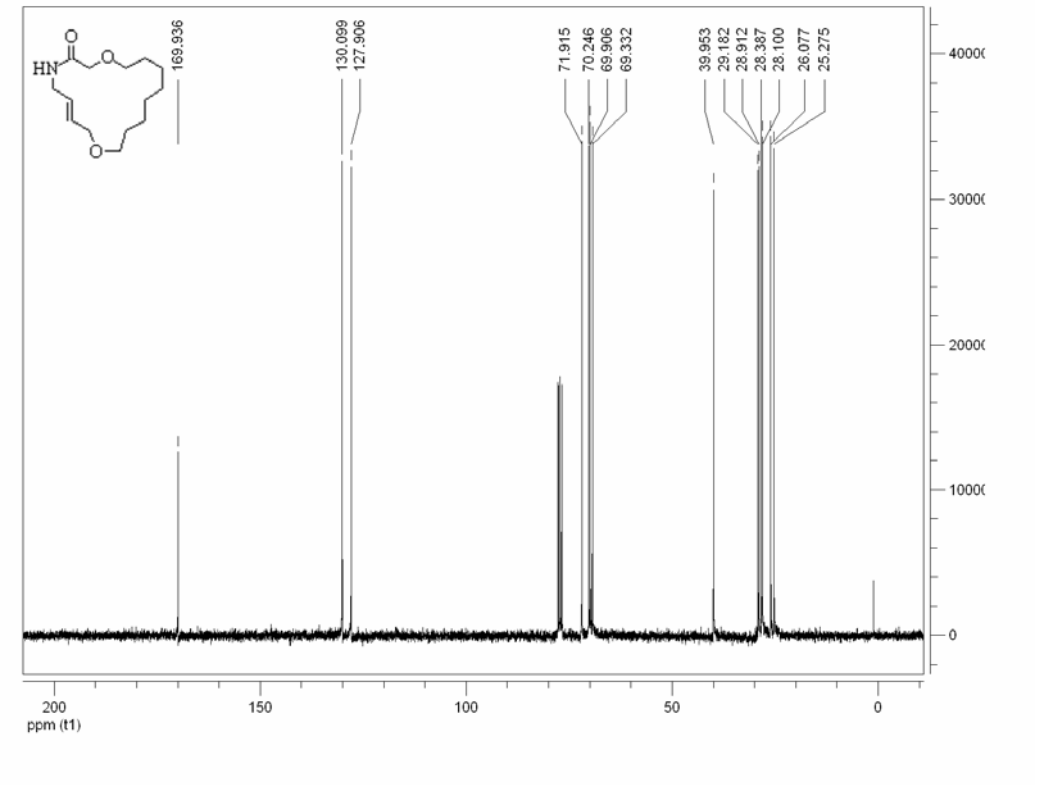
APPENDIX B
SELECTED NMR SPECTRAL DATA

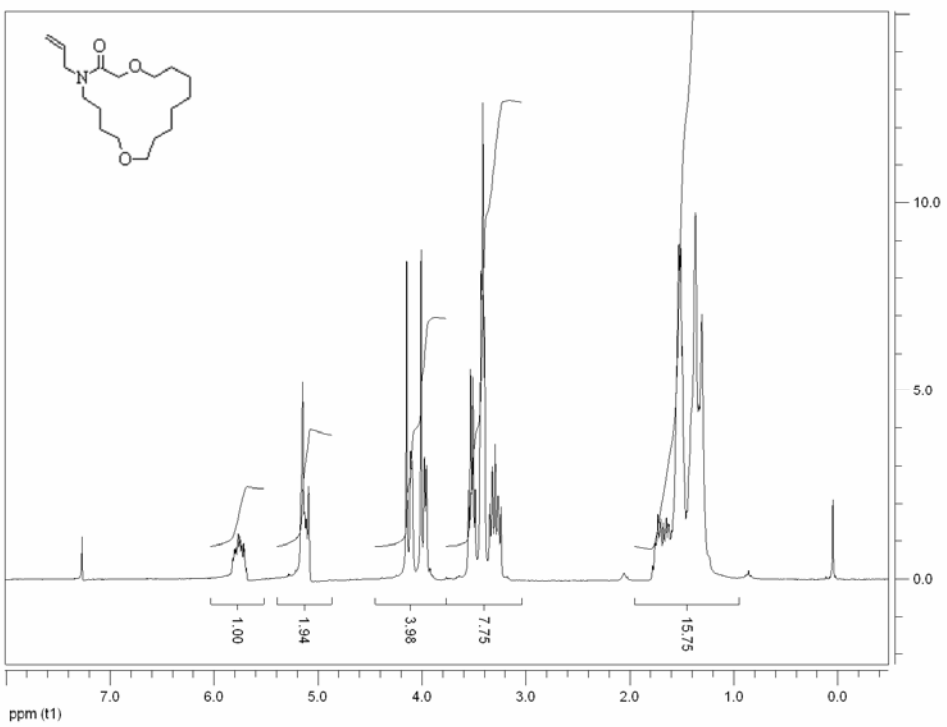
The ^{1}H and ^{13}C spectra of selected compounds from Chapters 2-5 are illustrated in this appendix. The spectra along with the proposed structure are shown.

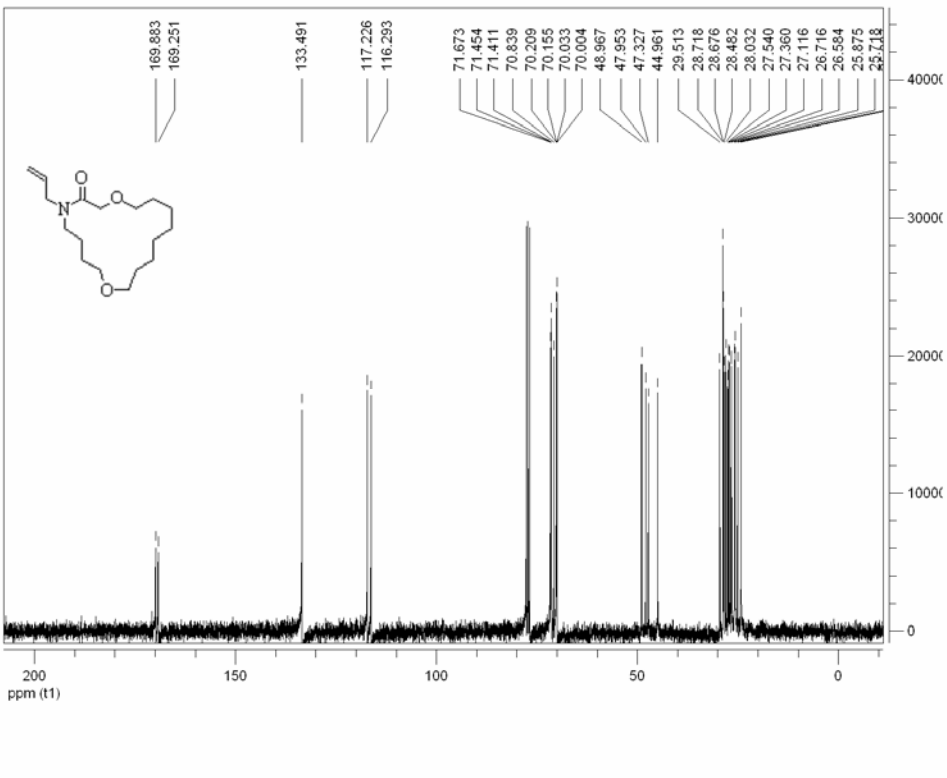

1.00
1.94
3.94
6.10
4.13
3.81
7.93
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)
250
200
150
100
50
0

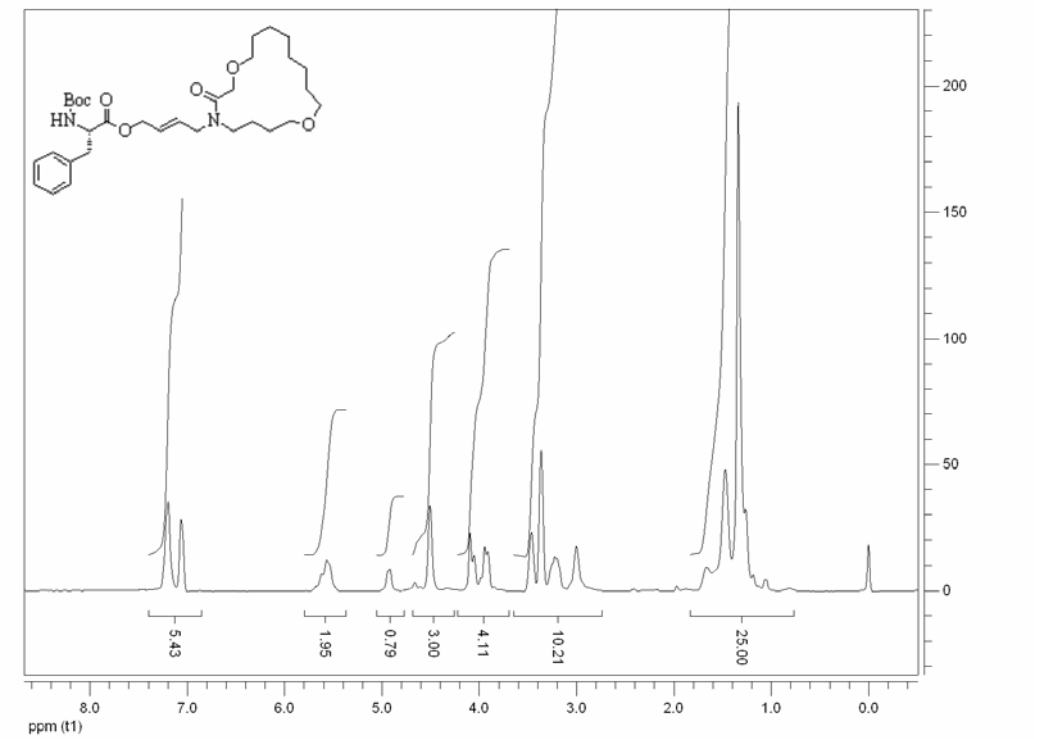

169.858
135.148
134.096
116.815
116.450
71.962
71.910
70.520
70.259
41.198
29.849
29.571
29.504
29.431
26.235
26.089
200
150
100
50
0
ppm (t1)
5000(
0

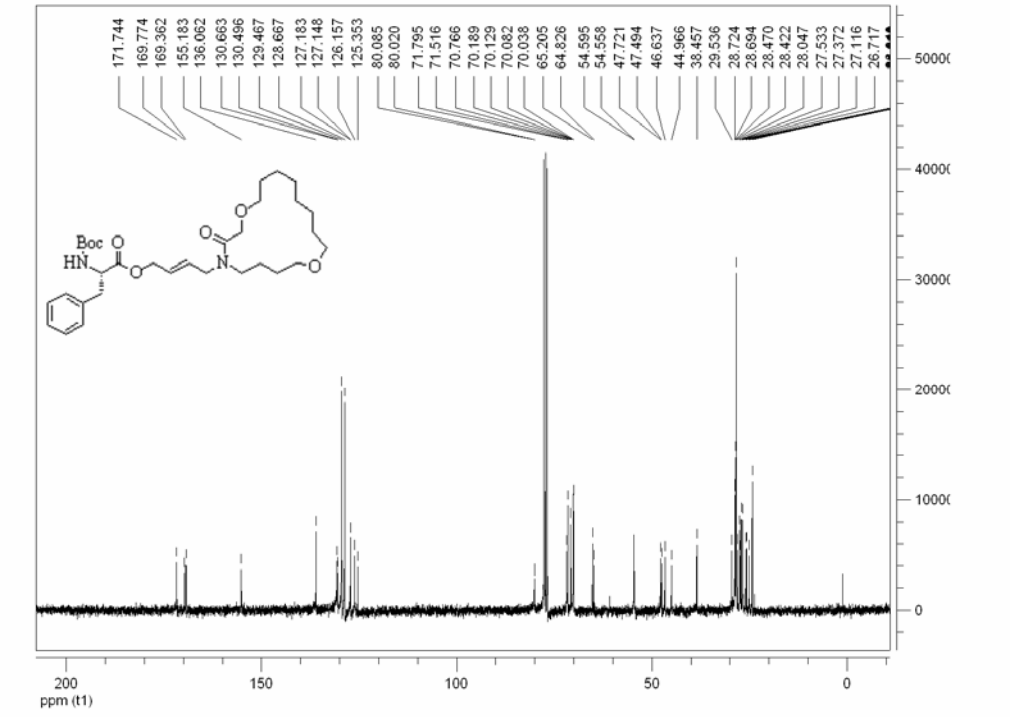

HN
O
O
O
150
100
50
0
1.00
1.96
6.13
4.18
11.97
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

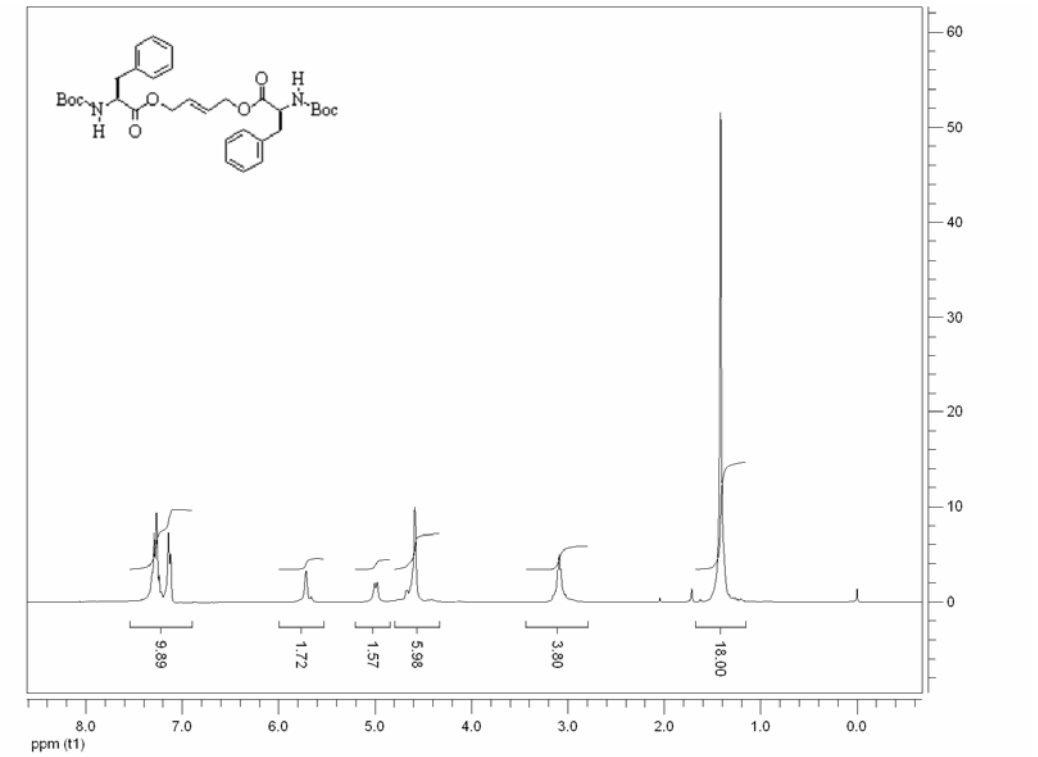

HN
O
O
O
169.936
130.099
127.906
71.915
70.246
69.906
69.332
39.953
29.182
28.912
28.387
28.100
26.077
25.275
40000
30000
20000
10000
0
200
150
100
50
0
ppm (t1)

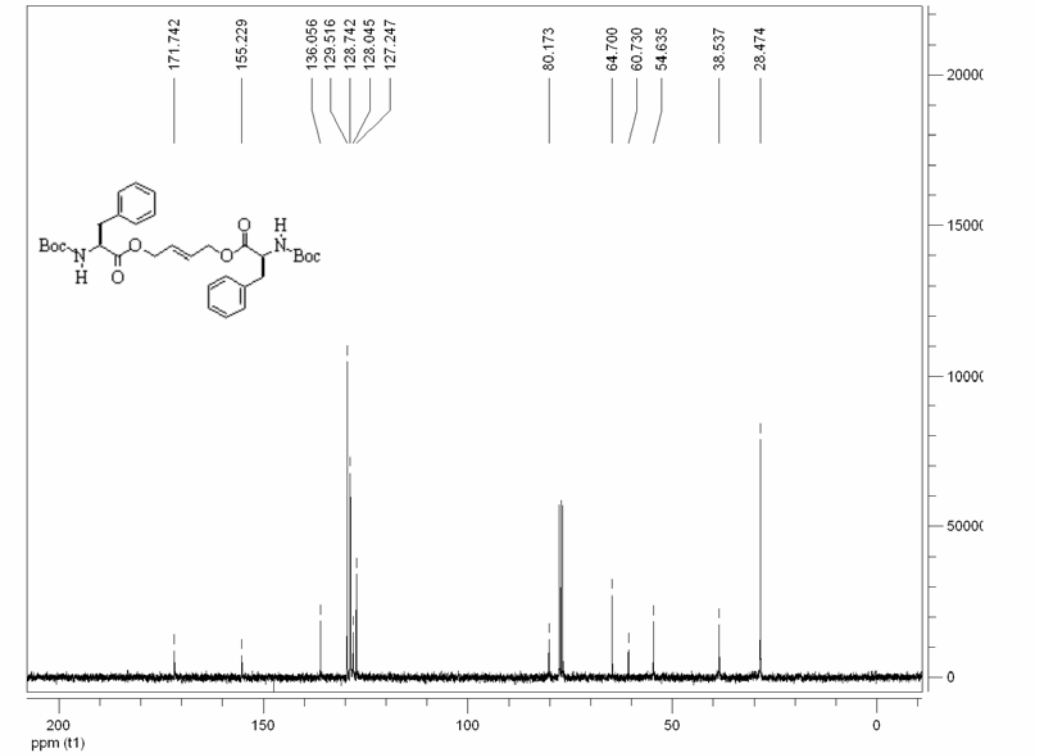

1.00
1.94
3.98
7.75
15.75
10.0
5.0
0.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

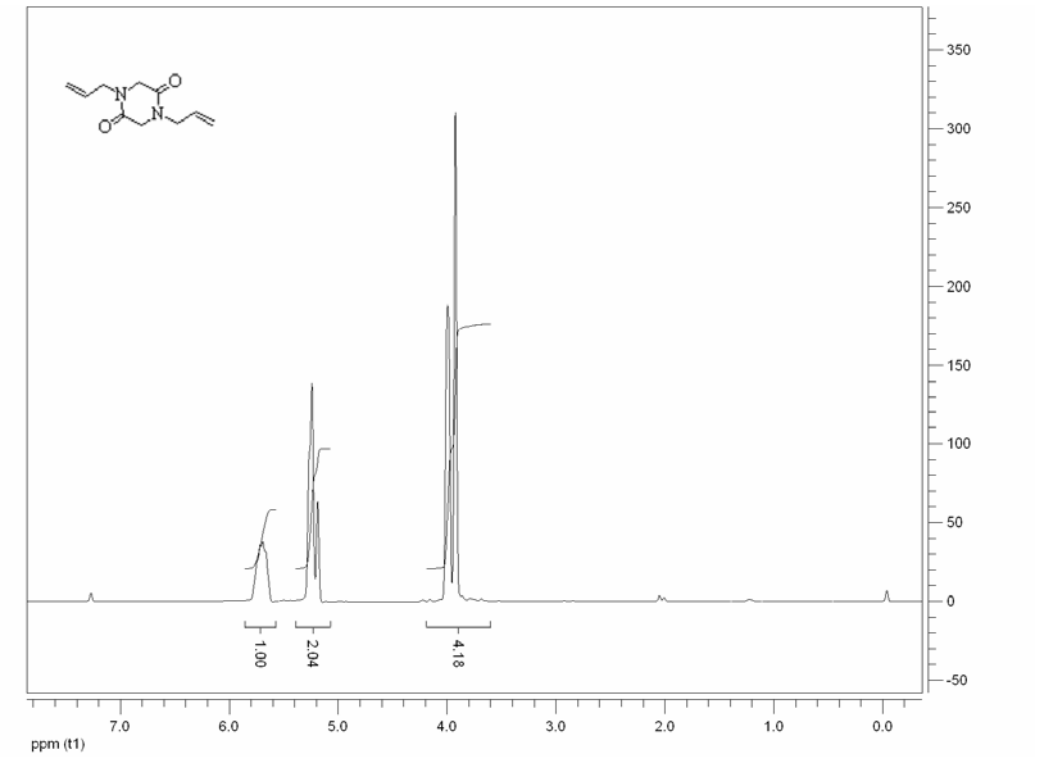

169.883
169.251
133.491
117.226
116.293
71.673
71.454
71.411
70.839
70.209
70.155
70.033
70.004
48.967
47.953
47.327
44.961
29.513
28.718
28.676
28.482
28.032
27.540
27.360
27.116
26.716
26.584
25.875
25.718
4000
3000
2000
1000
0
200
150
100
50
0
ppm (t1)

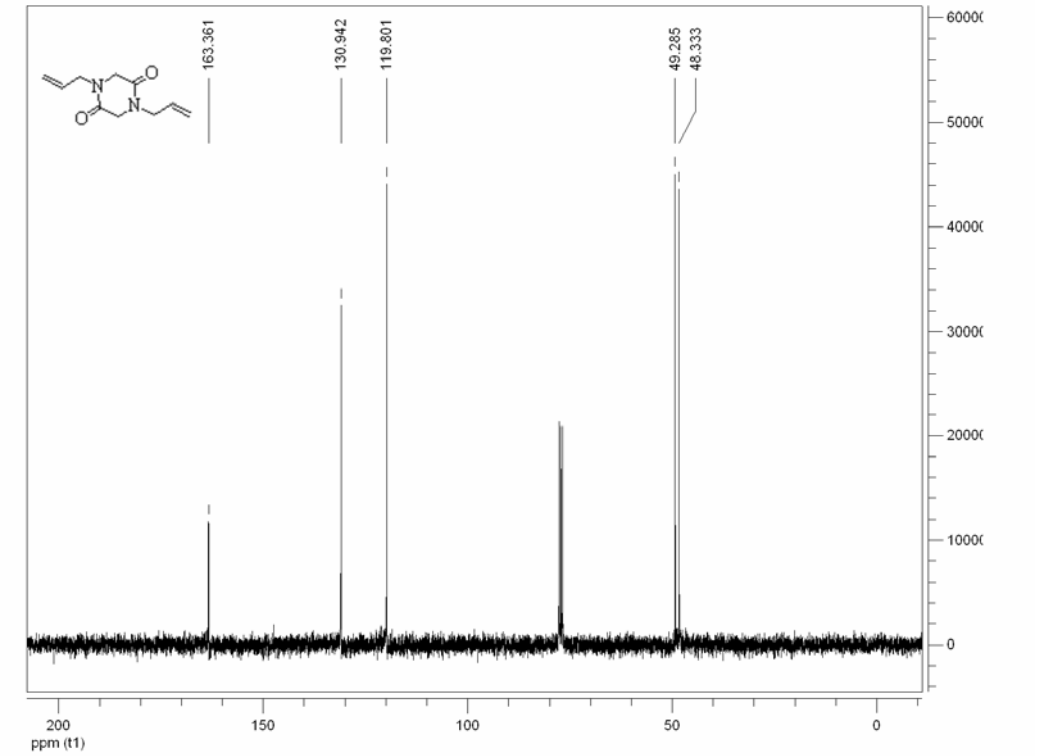

Boc
HN
O
O
O
O
N
O
5.43
1.95
0.79
3.00
4.11
10.21
25.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

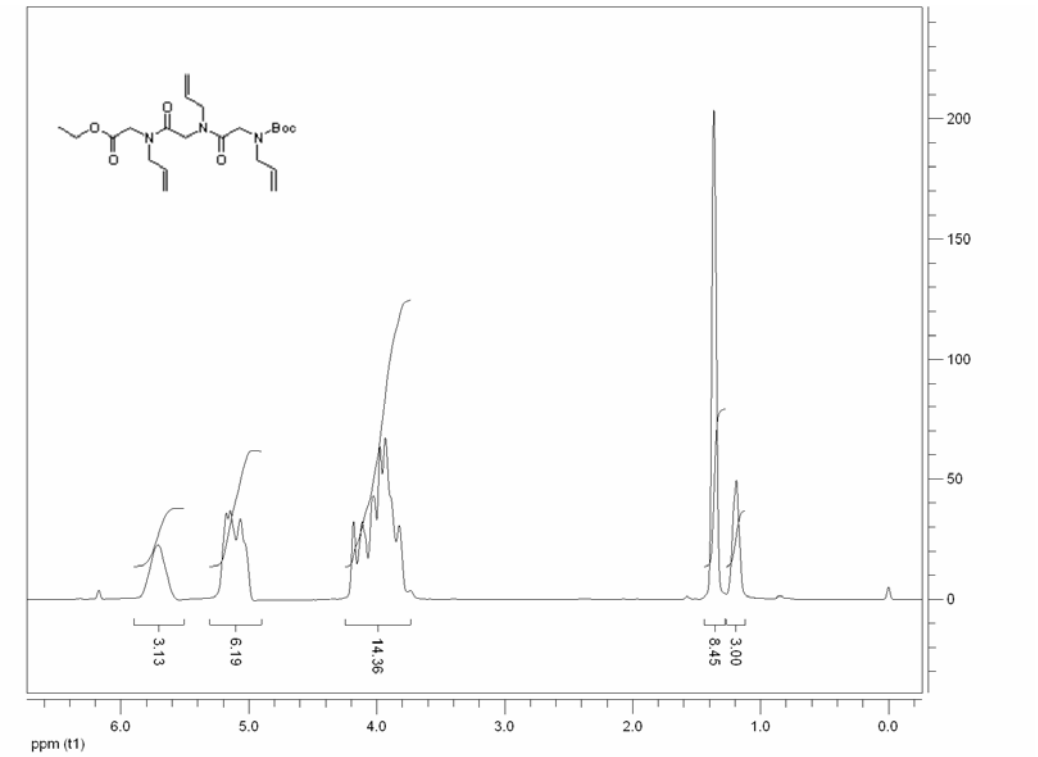

171.744
169.774
169.362
155.183
136.062
130.663
130.496
129.467
128.667
127.183
127.148
126.157
125.353
80.085
80.020
71.795
71.516
70.766
70.189
70.129
70.082
70.038
65.205
64.826
54.595
54.558
47.721
47.494
46.637
44.966
38.457
29.536
28.724
28.694
28.470
28.422
28.047
27.533
27.372
27.116
26.717
Boc
HN
O
O
O
O
N
O
200
150
100
50
0
ppm (t1)

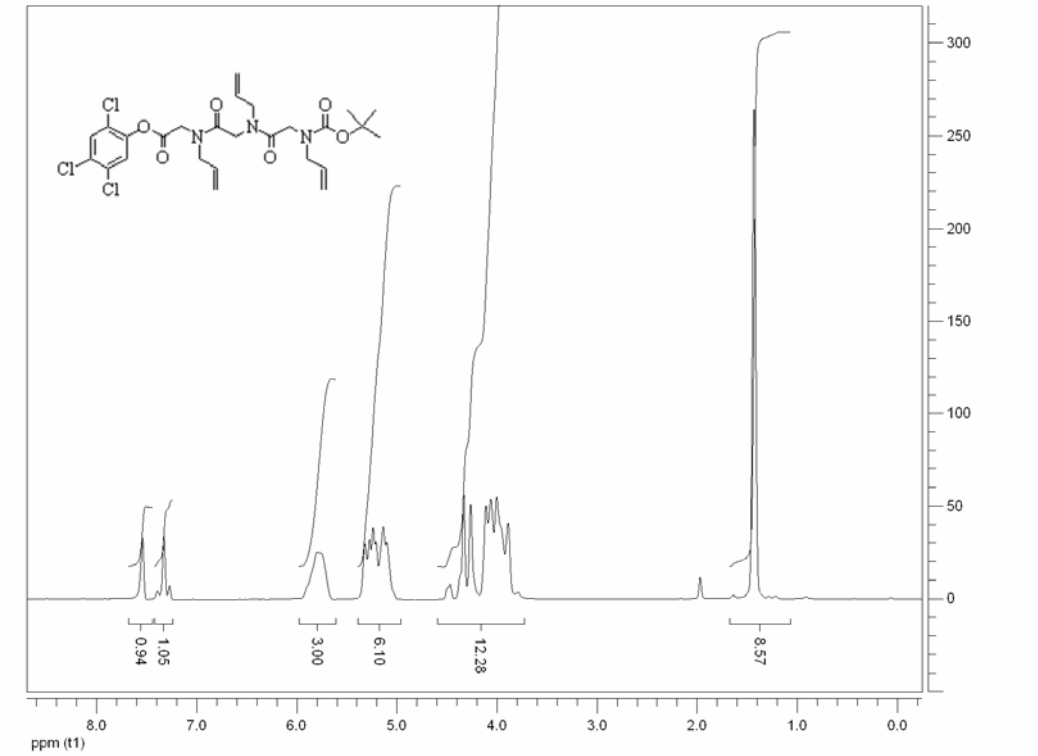

Boc
N
H
O
O
O
O
H
N
Boc
9.89
1.72
1.57
5.98
3.80
18.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

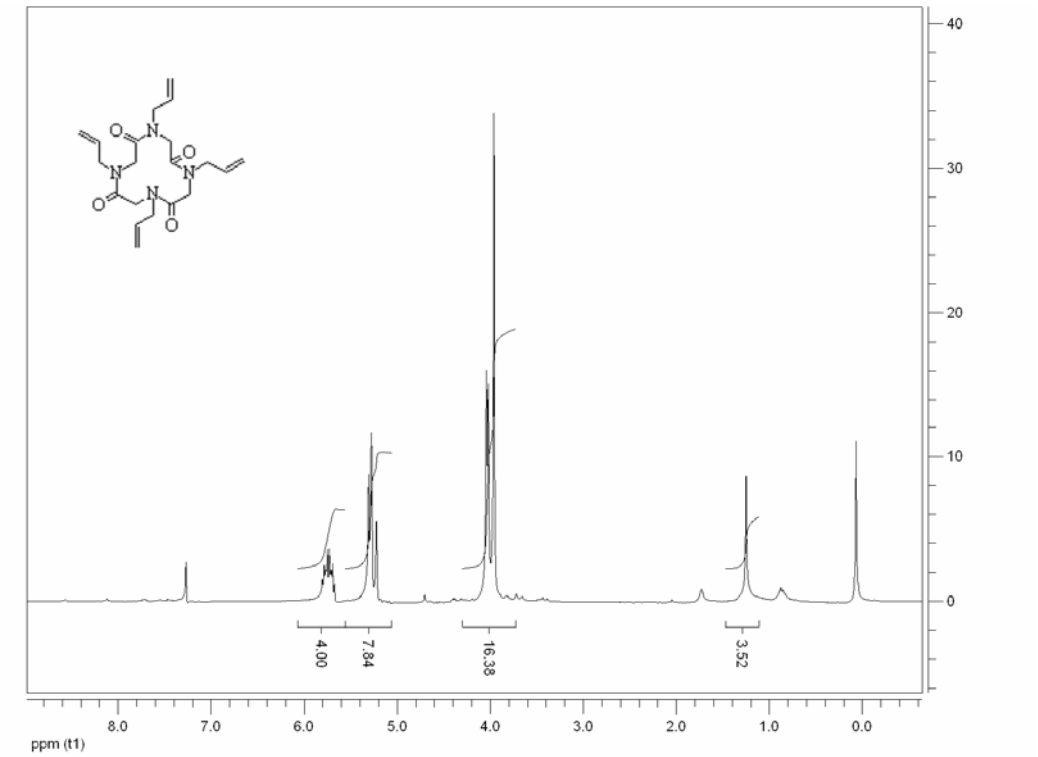

171.742
155.229
136.056
129.516
128.742
128.045
127.247
80.173
64.700
60.730
54.635
38.537
28.474
Boc
N
H
O
O
O
O
H
N
Boc
200
150
100
50
0
ppm (t1)

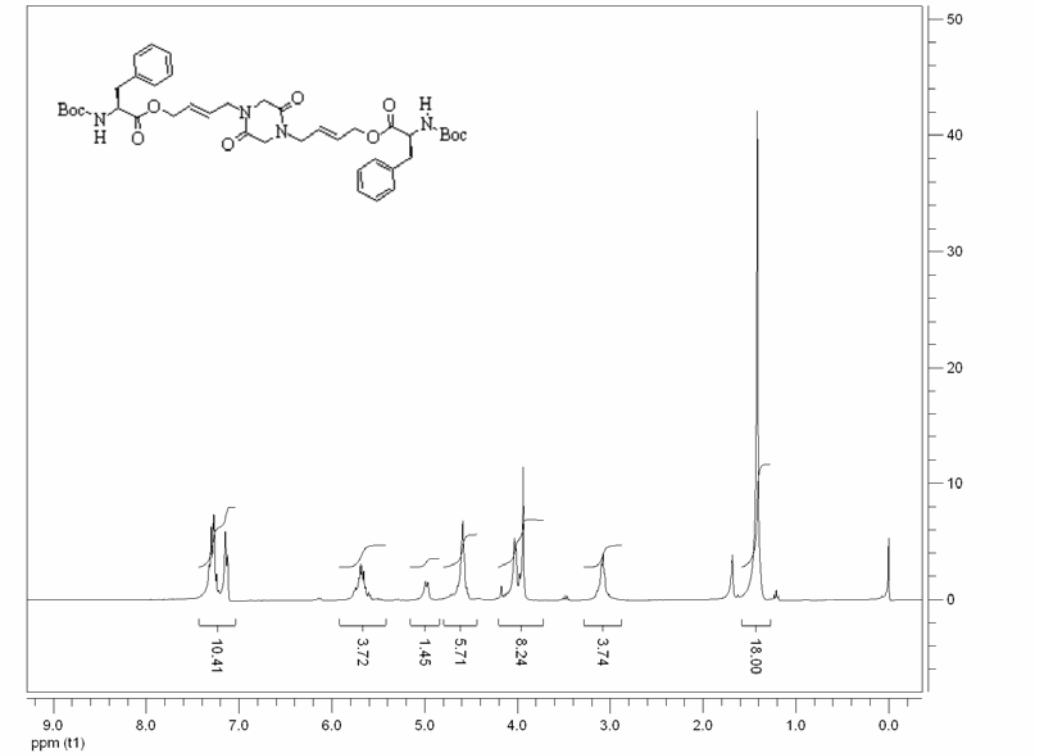

1.00
2.04
4.18
ppm (t1)

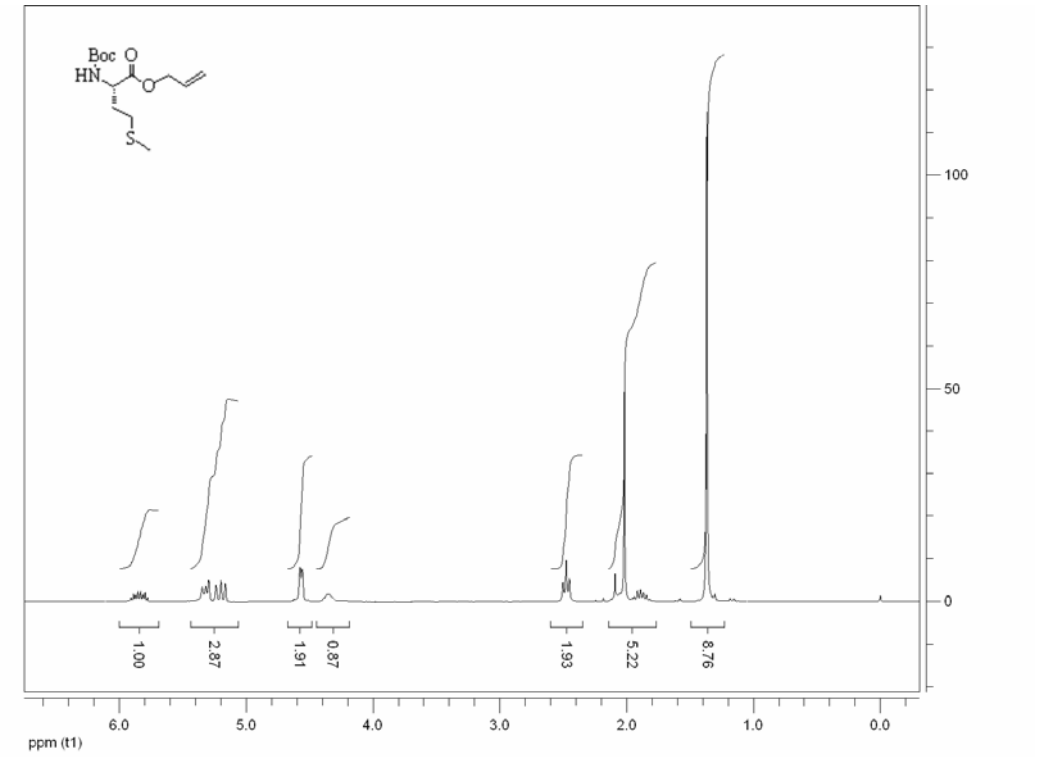

163.361
130.942
119.801
49.285
48.333
ppm (t1)

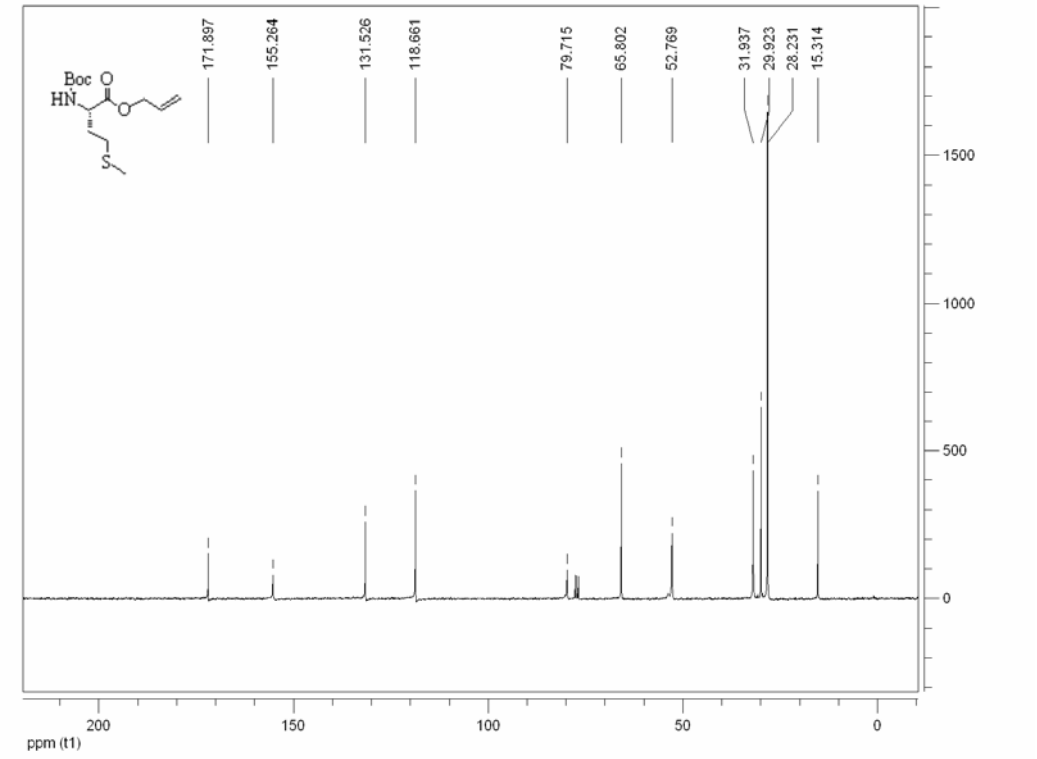

Boc
200
150
100
50
0
3.13
6.19
14.36
8.45
3.00
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

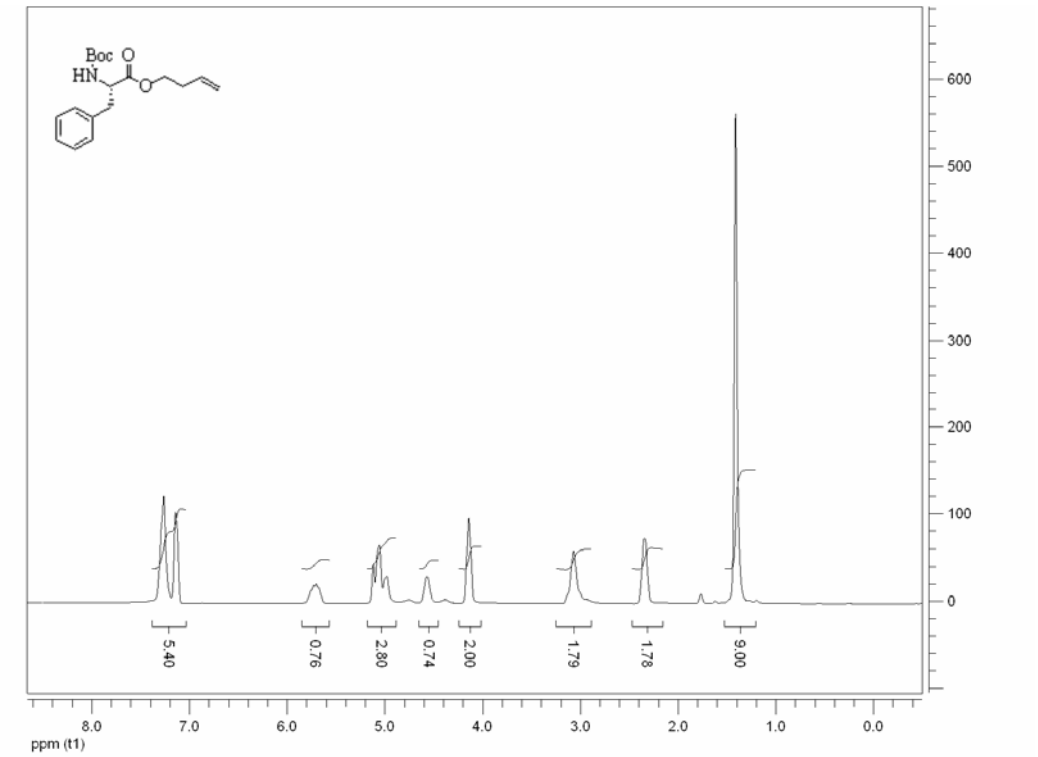

Cl
Cl
Cl
300
250
200
150
100
50
0
0.94
1.05
3.00
6.10
12.28
8.57
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

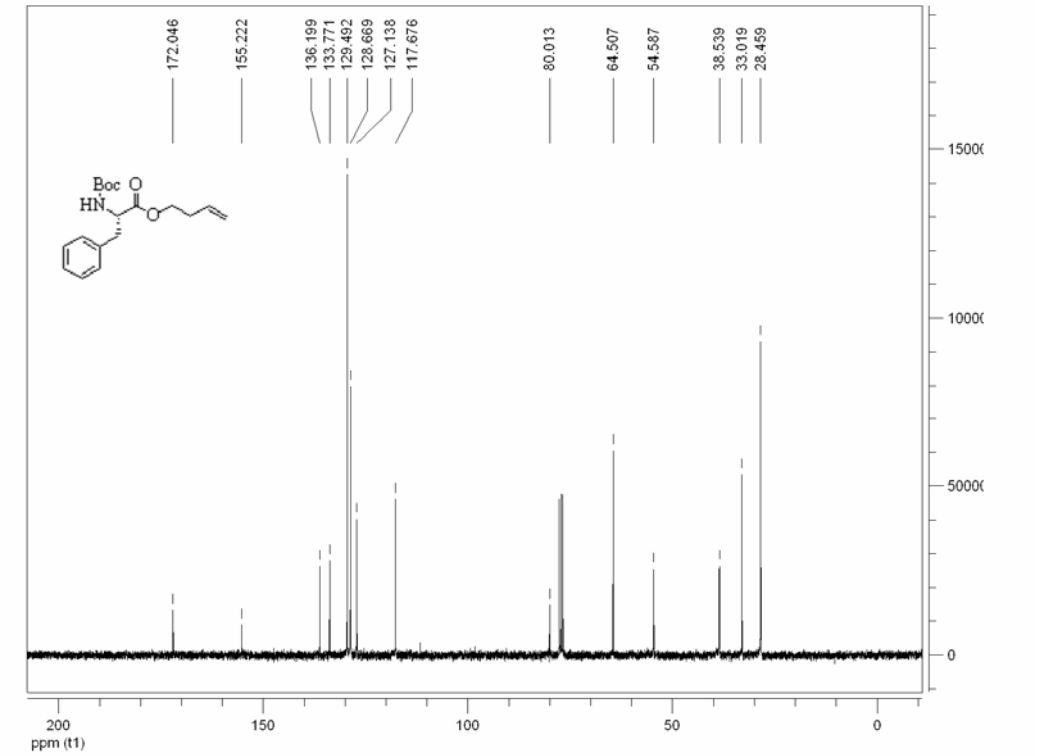

4.00
7.84
16.38
3.52
ppm (t1)

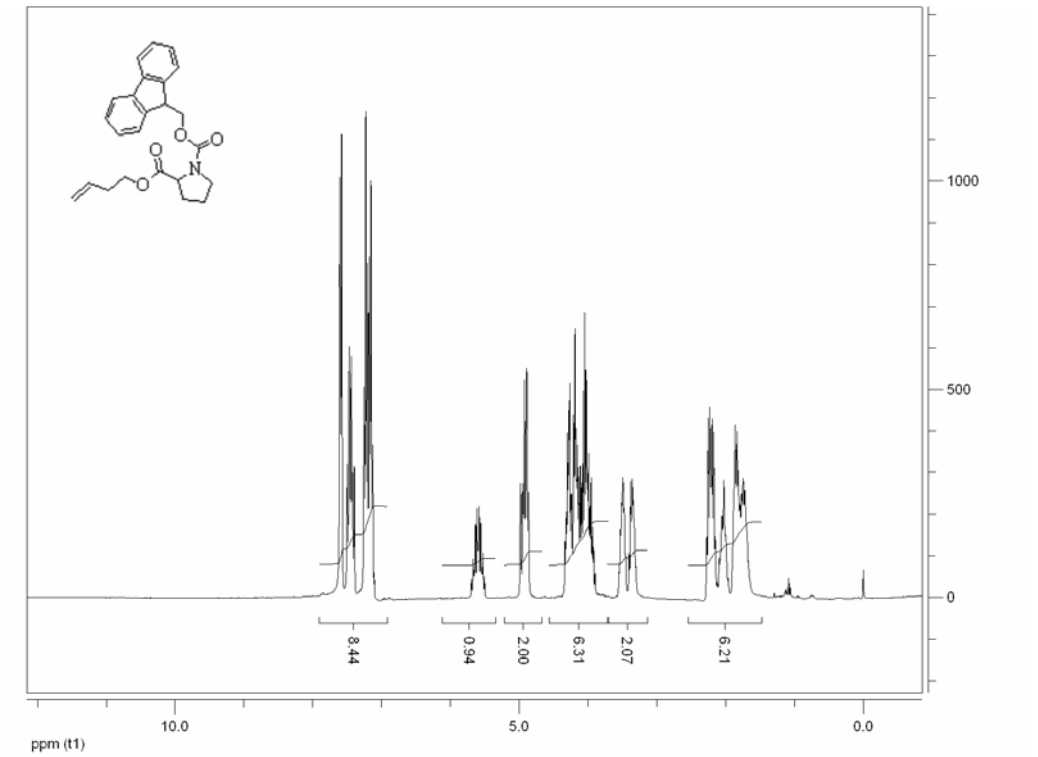

Boc
Boc
10.41
3.72
1.45
5.71
8.24
3.74
18.00
ppm (t1)

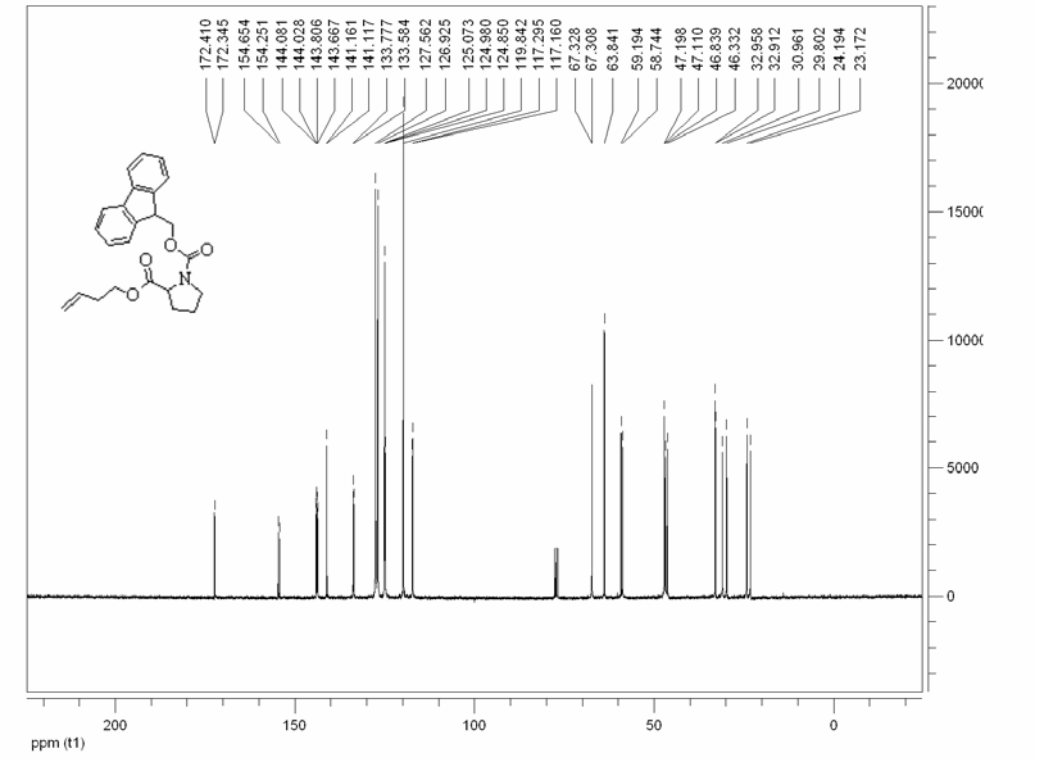

Boc
O
HN
O
S
100
50
0
1.00
2.87
1.91
0.87
1.93
5.22
8.76
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

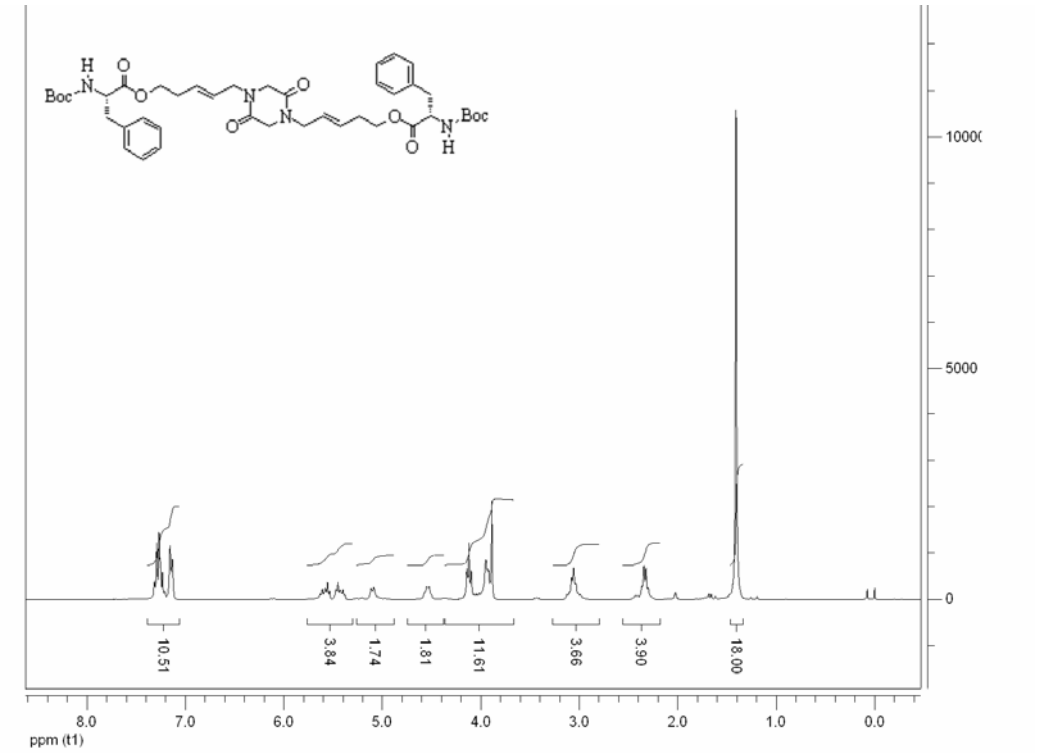

Boc
O
HN
O
S
171.897
155.264
131.526
118.661
79.715
65.802
52.769
31.937
29.923
28.231
15.314
1500
1000
500
0
200
150
100
50
0
ppm (t1)

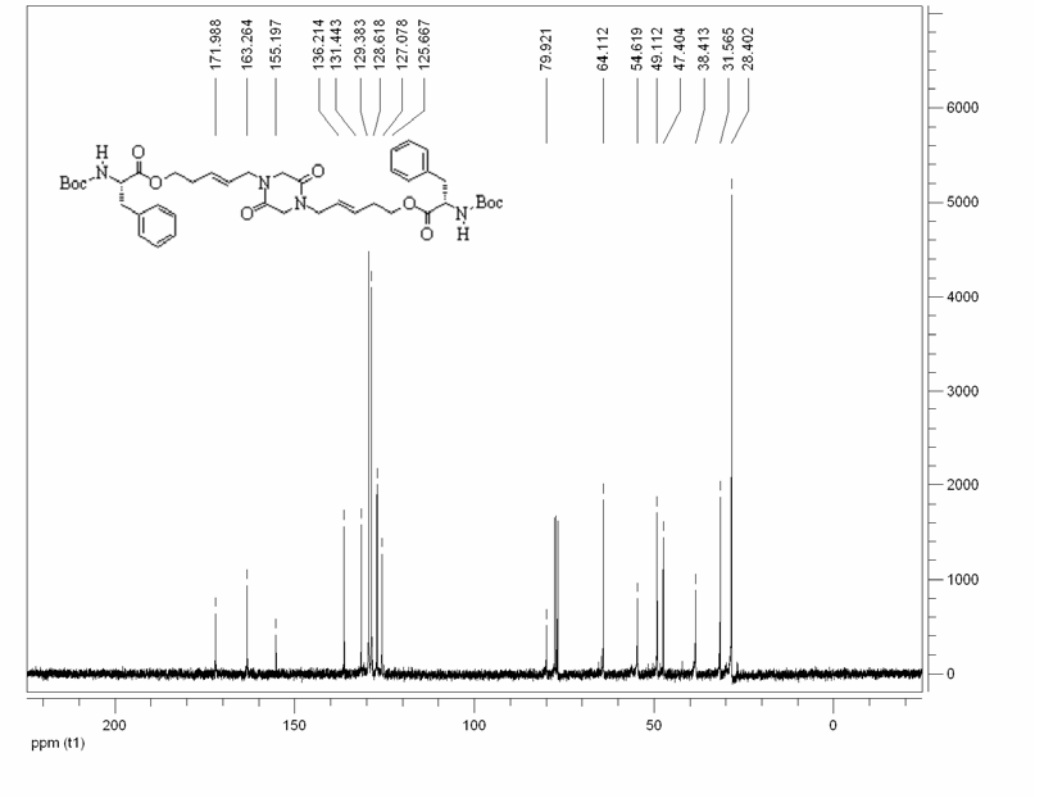

Boc
O
HN
O
600
500
400
300
200
100
0
5.40
0.76
2.80
0.74
2.00
1.79
1.78
9.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

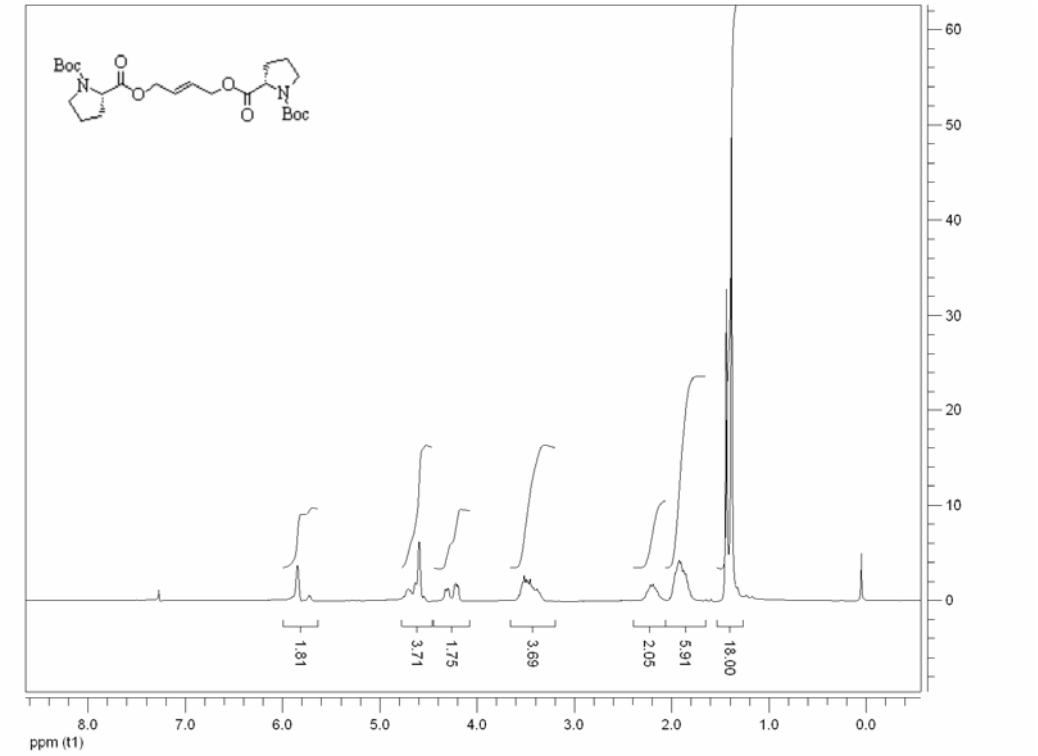

172.046
155.222
136.199
133.771
129.492
128.669
127.138
117.676
80.013
64.507
54.587
38.539
33.019
28.459
Boc
O
HN
O
15000
10000
50000
0
200
150
100
50
0
ppm (t1)

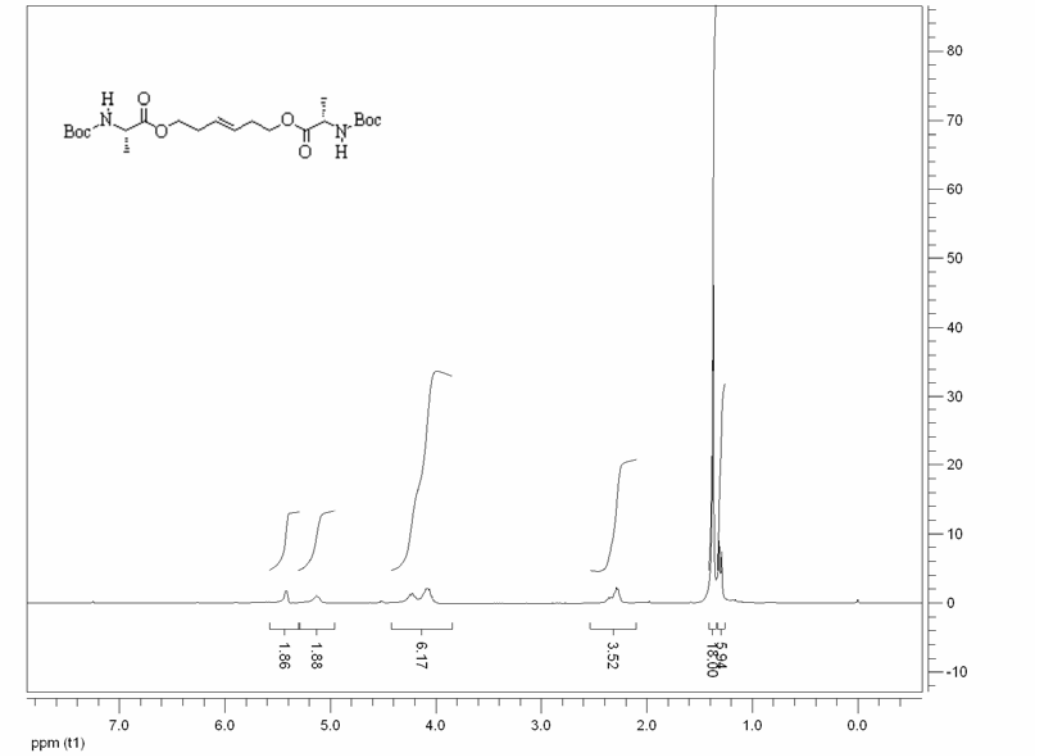

1000
500
0
8.44
0.94
2.00
6.31
2.07
6.21
10.0
5.0
0.0
ppm (t1)

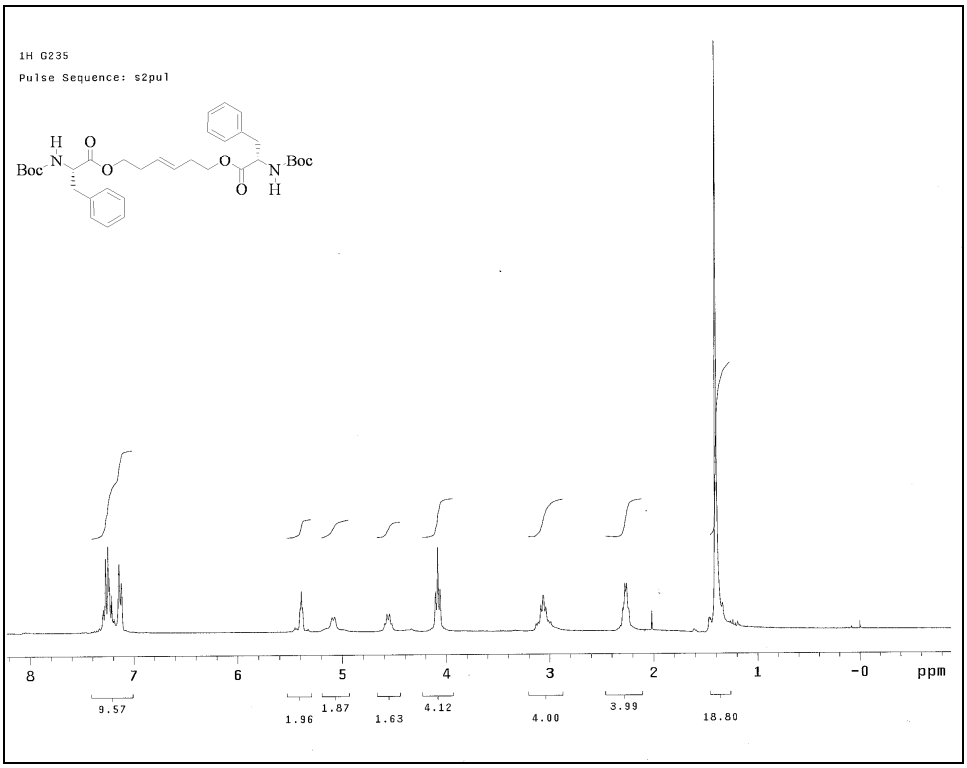

172.410
172.345
154.654
154.251
144.081
144.028
143.806
143.667
141.161
141.117
133.777
133.584
127.562
126.925
125.073
124.980
124.850
119.842
117.295
117.160
67.328
67.308
63.841
59.194
58.744
47.198
47.110
46.839
46.332
32.958
32.912
30.961
29.802
24.194
23.172
20000
15000
10000
5000
0
200
150
100
50
0
ppm (t1)

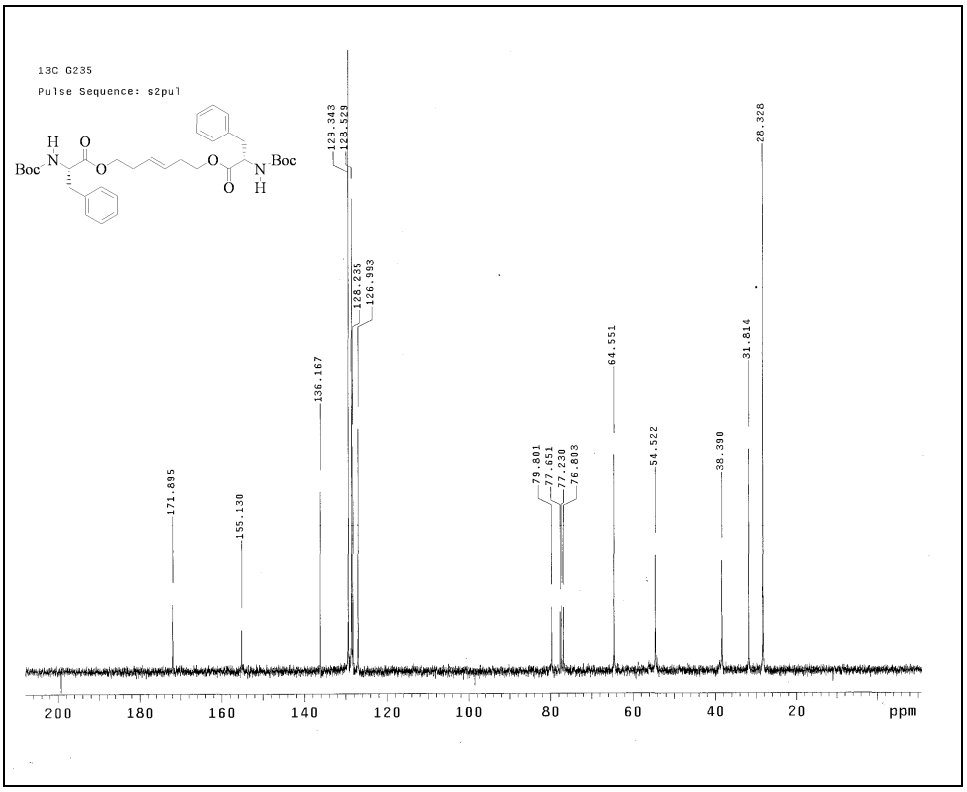

Boc
Boc
10000
5000
0
10.51
3.84
1.74
1.81
11.61
3.66
3.90
18.00
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm (t1)

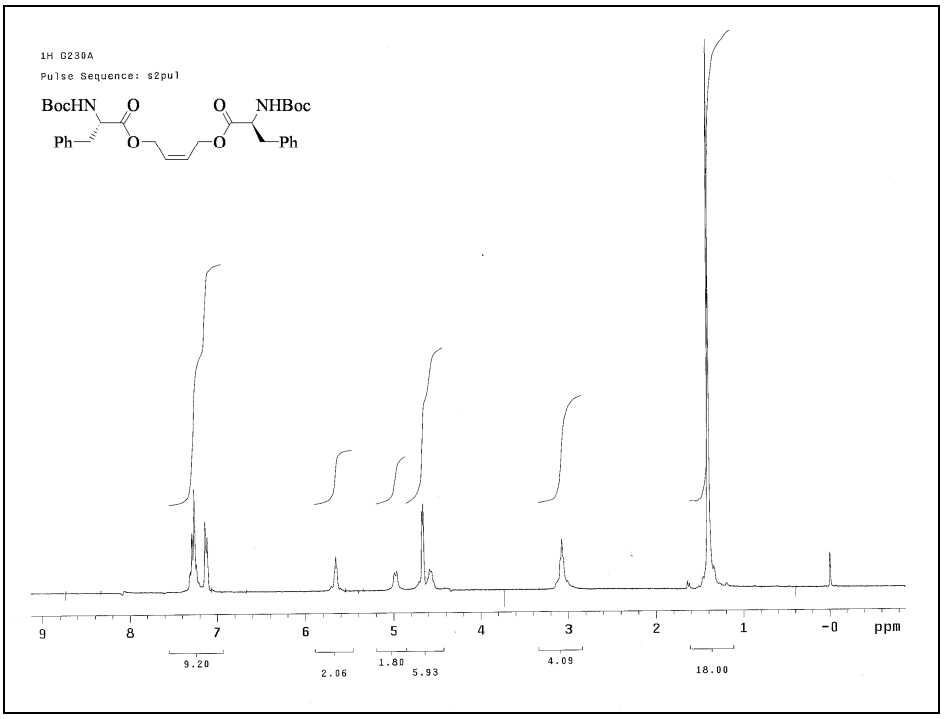

171.988
163.264
155.197
136.214
131.443
129.383
128.618
127.078
125.667
79.921
64.112
54.619
49.112
47.404
38.413
31.565
28.402
Boc
Boc
6000
5000
4000
3000
2000
1000
0
200
150
100
50
0
ppm (t1)

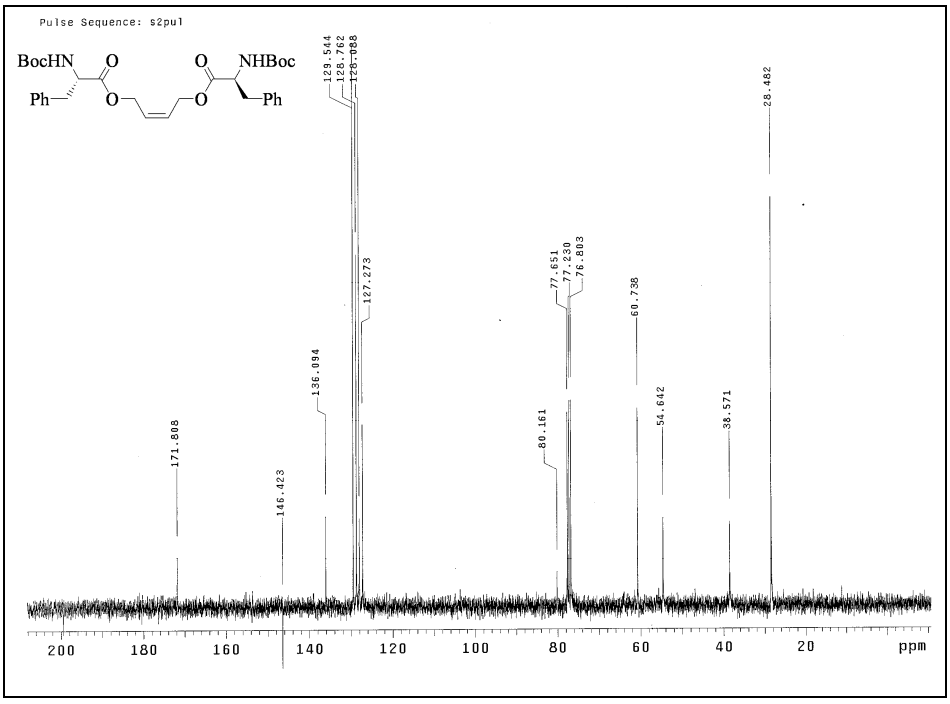

Boc
Boc
1.81
3.71
1.75
3.69
2.05
5.91
18.00
ppm (t1)

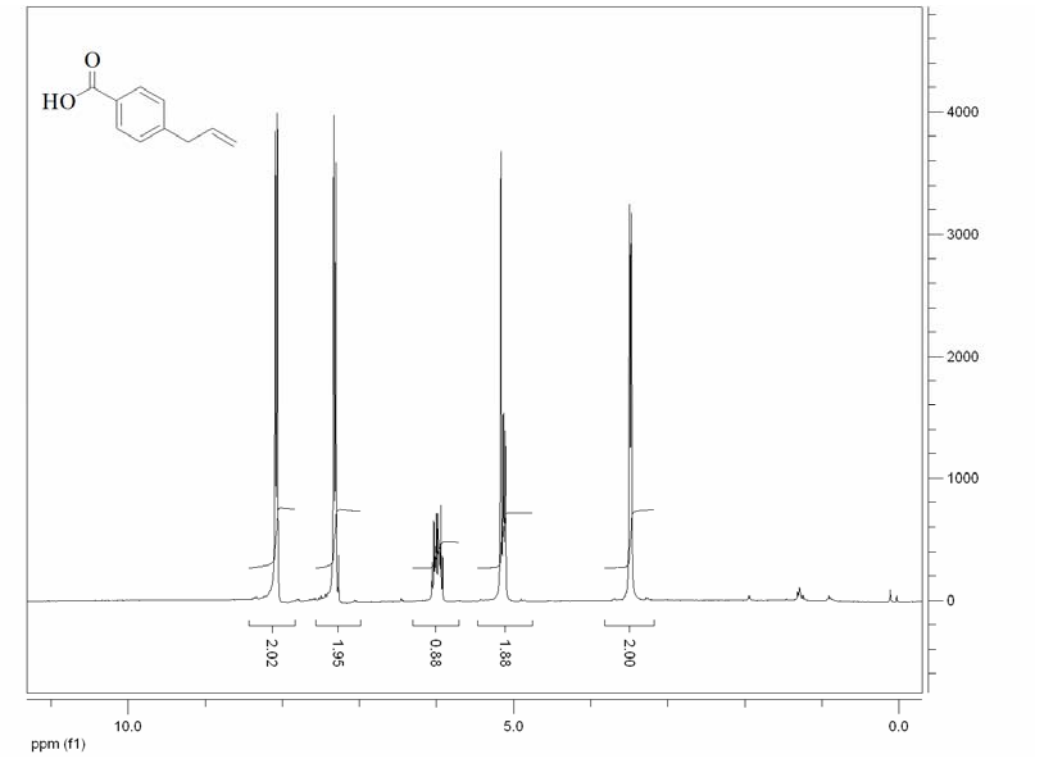

Boc
Boc
1.86
1.88
6.17
3.52
5.84
18.00
ppm (t1)

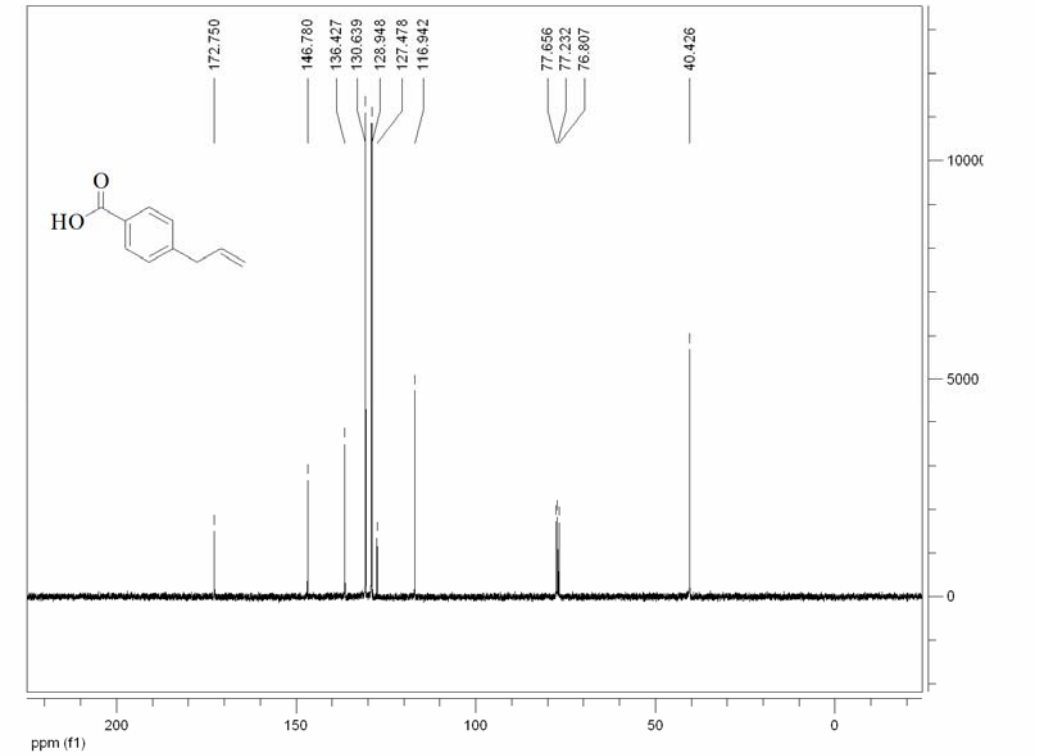

1H G235
Pulse Sequence: s2pul
Boc
Boc
8
7
6
5
4
3
2
1
-0
ppm
9.57
1.96
1.87
1.63
4.12
4.00
3.99
18.80

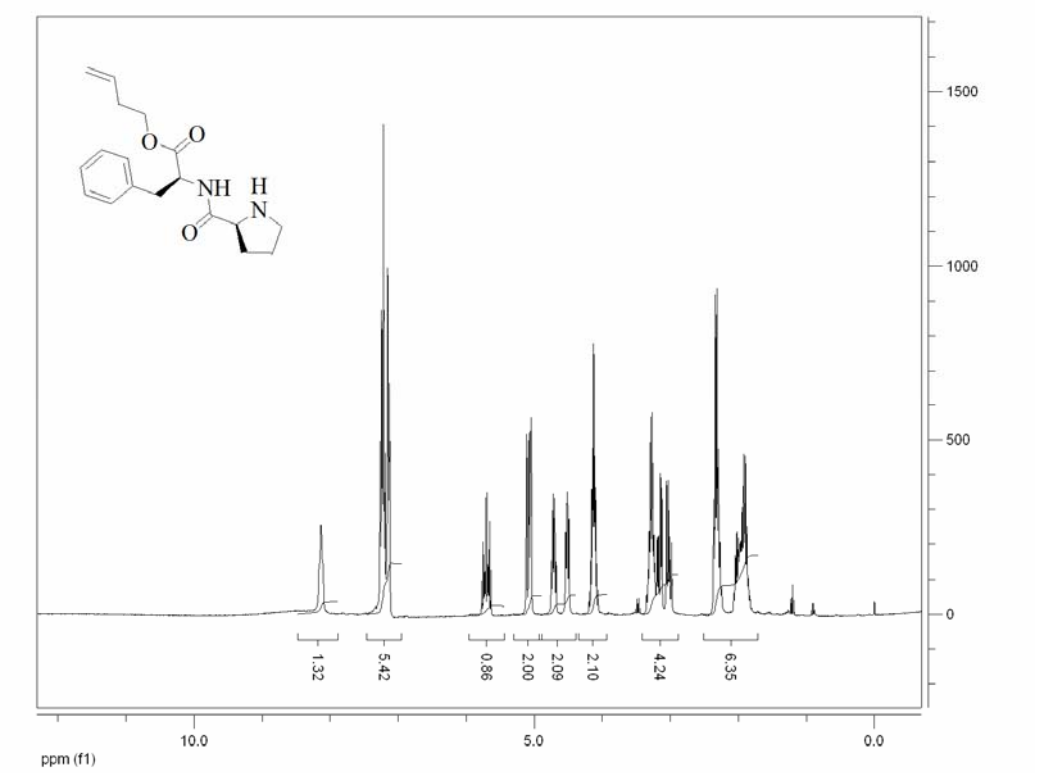

13C G235
Pulse Sequence: s2pul
Boc
Boc
123.343
123.529
128.235
126.993
136.167
171.895
155.130
79.801
77.651
77.230
76.803
64.551
54.522
38.390
31.814
28.328
200
180
160
140
120
100
80
60
40
20
ppm

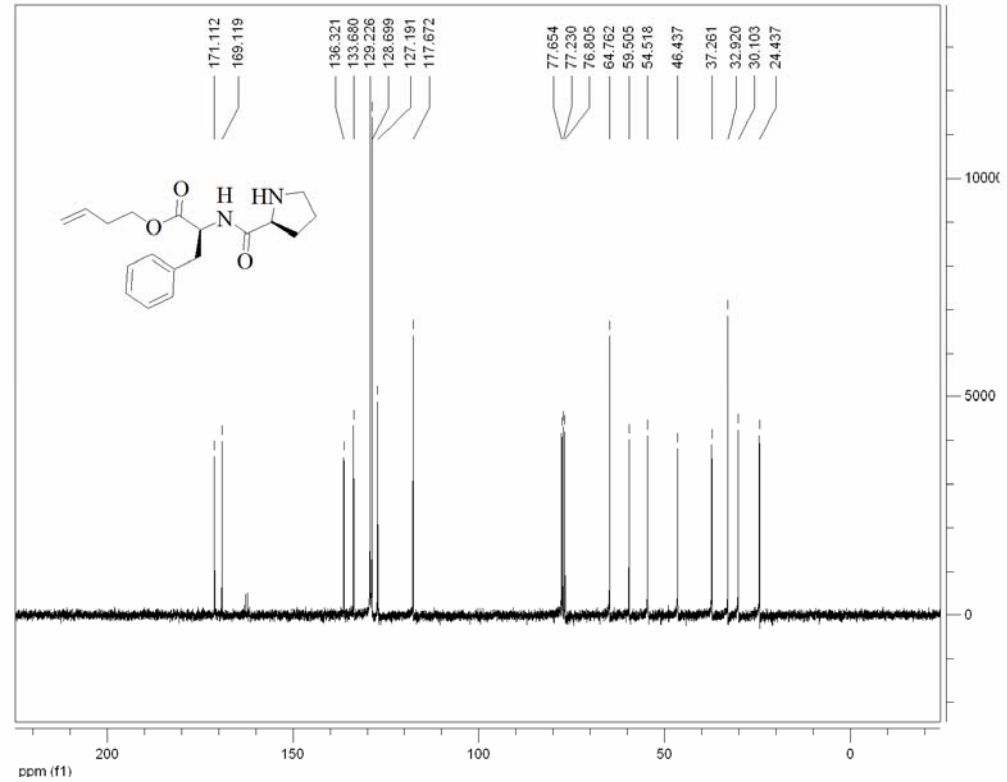

1H G230A
Pulse Sequence: s2pul
BocHN
NHBoc
Ph
Ph
9
8
7
6
5
4
3
2
1
-0
ppm
9.20
2.06
1.80
5.93
4.09
18.00

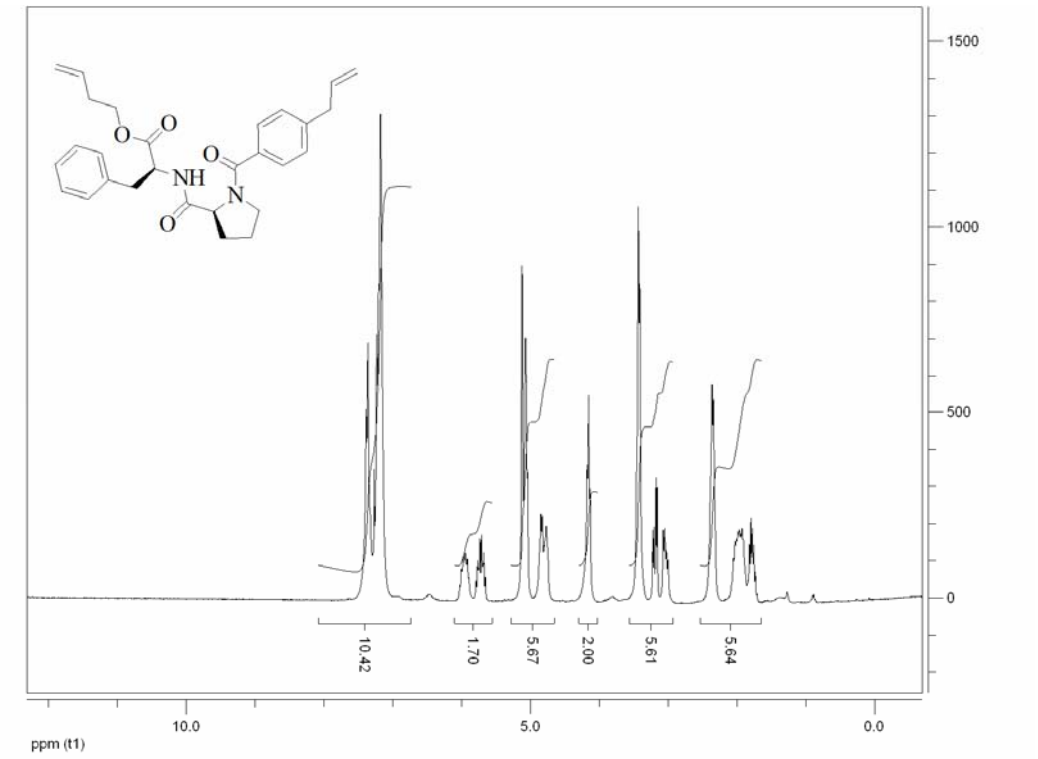

Pulse Sequence: s2pul
BocHN
NHBoc
Ph
Ph
129.544
128.762
128.088
127.273
136.084
171.808
146.423
80.161
77.651
77.230
76.803
60.738
54.642
38.571
28.482
200
180
160
140
120
100
80
60
40
20
ppm

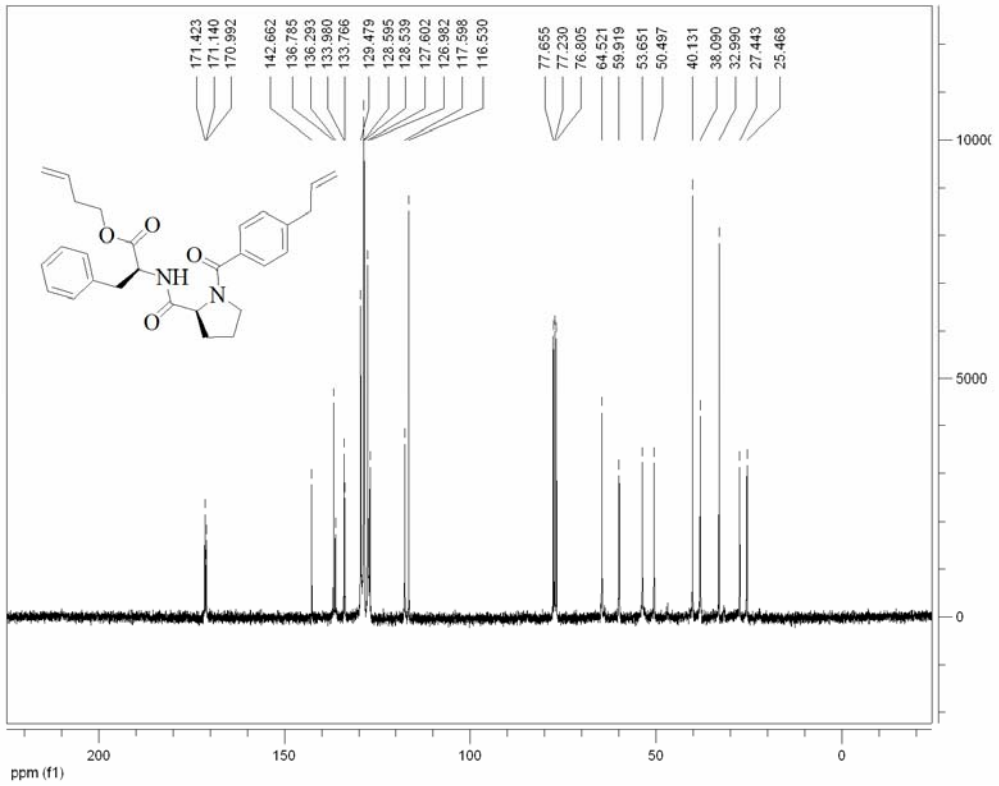

HO
O
4000
3000
2000
1000
0
2.02
1.95
0.88
1.88
2.00
10.0
5.0
0.0
ppm (f1)

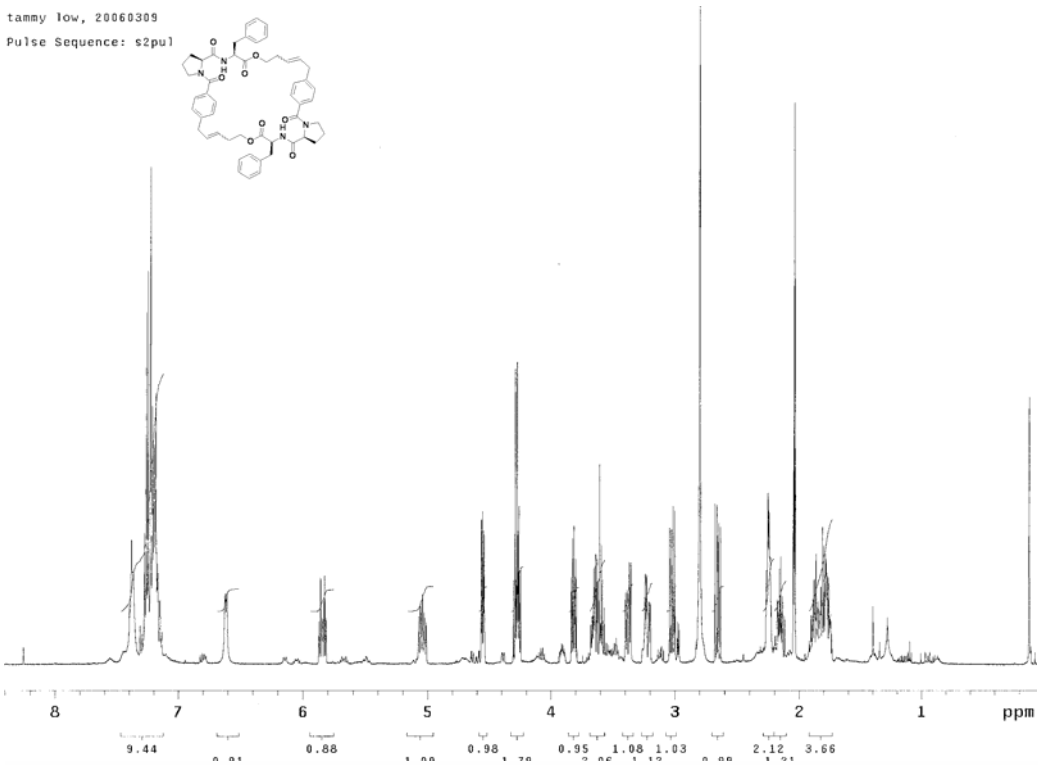

172.750
146.780
136.427
130.639
128.948
127.478
116.942
77.656
77.232
76.807
40.426
HO
O
10000
5000
0
200
150
100
50
0
ppm (f1)

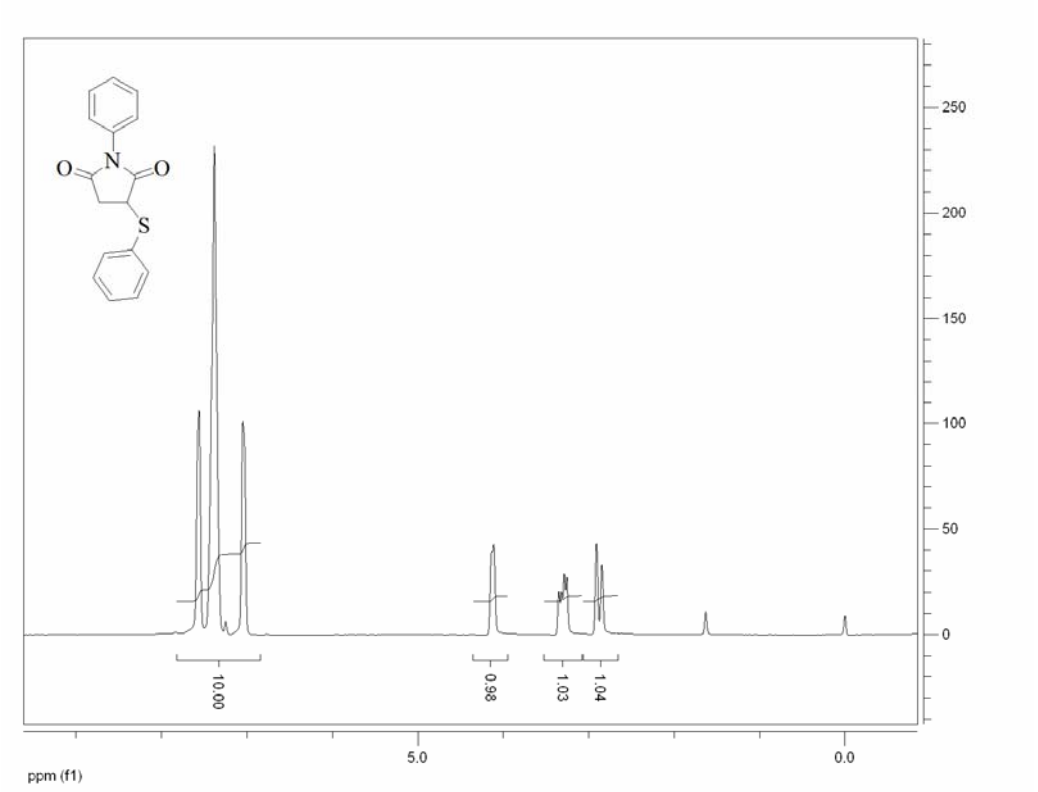

1500
1000
500
0
1.32
5.42
0.86
2.00
2.09
2.10
4.24
6.35
10.0
5.0
0.0
ppm (f1)

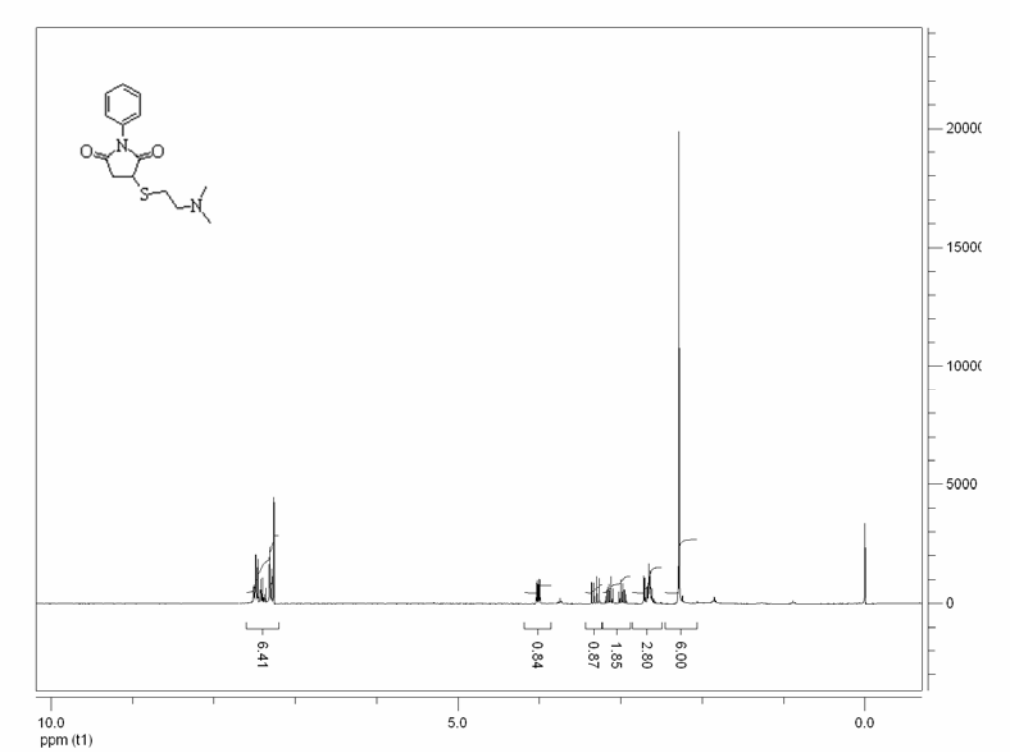

171.112
169.119
136.321
133.680
129.226
128.699
127.191
117.672
77.654
77.230
76.805
64.762
59.505
54.518
46.437
37.261
32.920
30.103
24.437
10000
5000
0
200
150
100
50
0
ppm (f1)

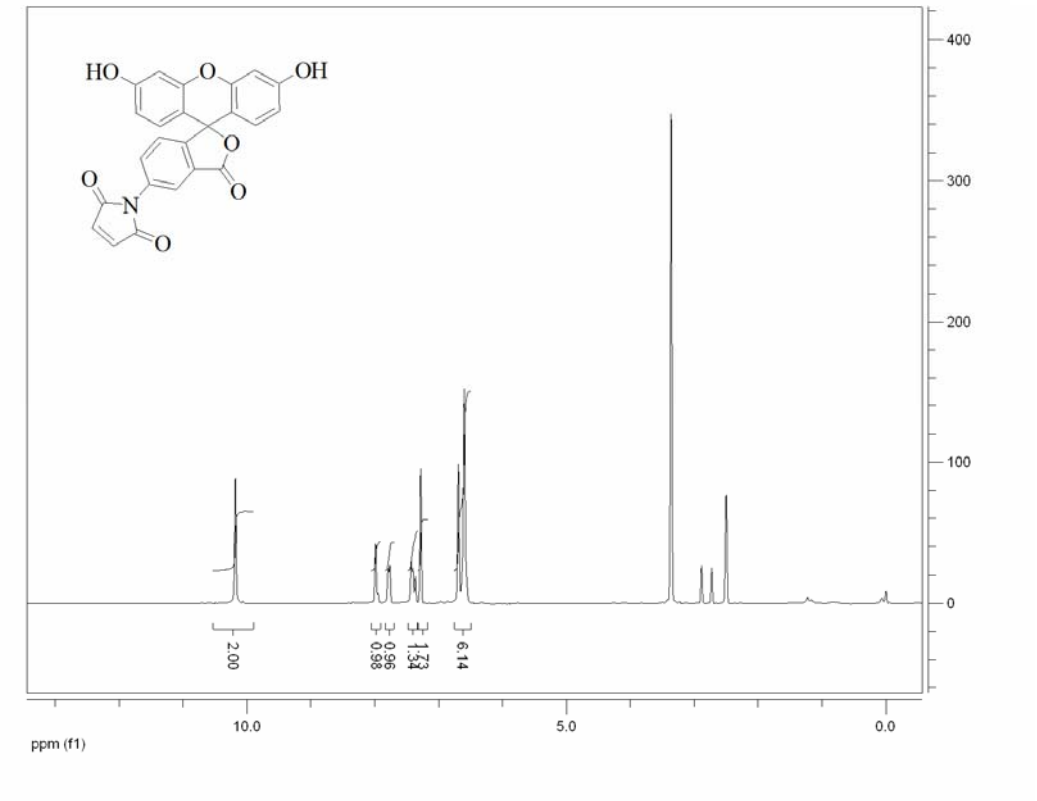

1500
1000
500
0
10.42
1.70
5.67
2.00
5.61
5.64
10.0
5.0
0.0
ppm (t1)


171.423
171.140
170.992
142.662
136.785
136.293
133.980
133.766
129.479
128.595
128.539
127.602
126.982
117.598
116.530
77.655
77.230
76.805
64.521
59.919
53.651
50.497
40.131
38.090
32.990
27.443
25.468
10000
5000
0
200
150
100
50
0
ppm (f1)


tammy low, 20060309
Pulse Sequence: s2pul
8
7
6
5
4
3
2
1
ppm
9.44
0.88
0.98
0.95
1.08
1.03
2.12
3.66


O
N
O
S
250
200
150
100
50
0
10.00
0.98
1.03
1.04
5.0
0.0
ppm (f1)


6.41
0.84
0.87
1.85
2.80
6.00
10.0
5.0
0.0
ppm (t1)


HO
O
OH
O
O
N
O
O
2.00
0.96
0.98
1.53
1.54
6.14
10.0
5.0
0.0
ppm (f1)

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BIOGRAPHICAL SKETCH

Tammy K. C. Low was born in Taiwan ROC. As the daughter of a career Air Force Non Commissioned Officer, she grew up in California, Nebraska, North Carolina, Taiwan, and Okinawa, Japan. After graduating from Eastern Wayne High School, Goldsboro, North Carolina in 1993, she accepted an Air Force ROTC scholarship and attended North Carolina State University. Tammy was awarded a Bachelor of Science degree in biochemistry and her commission as a second lieutenant in the Air Force in May 1997.

The United States Air Force Academy (USAFA) selected Tammy to pursue her Master of Science degree in chemistry. She attended the University of Illinois at Urbana-Champaign and graduated in August 1998. Tammy was then assigned to Patrick Air Force Base (AFB), Florida, where she held the position of Biotechnology Research Officer, involved with treaty monitoring and counter proliferation of nuclear, chemical, and biological weapons. Her next assignment took her to the Department of Chemistry at the USAFA, Colorado Springs, Colorado, where she taught general, organic, and biochemistry, lectured in chemistry of weapons, and researched on ionic liquids and nucleic acid derivatives. She discovered her joy of teaching at the Academy, and to continue her teaching aspirations, Tammy accepted the Air Force Ph.D. program. She moved to Gainesville, Florida in August 2003, to study organic chemistry under Dr. Eric Enholm at the University of Florida.